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STUDIES ON THE INHALATION TOXICITY OF DYES PRESENT IN COLORED SMOKE MUNITIONS
FINAL REPORT FOR PHASE II STUDIES: RANGE FINDING AND TOXICOKINETICS STUDIES OF INHALED DYE AEROSOLS

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U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Project Order 3807

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The USAMBRDL has an interest in the potential inhalation toxicity of yellow dye (SY) and a yellow/green dye mixture (SY/SG) used in colored smoke munitions. In the rangefinding studies, no mortalities or life-threatening lesions were observed in rats exposed for as long as 6 hours per day for 5 days to airborne concentrations greater than 1 g/m^3 of either SY or SY/SG. The particle size of the aerosols employed was about 5 µm MMAD. In the toxicokinetic studies, rats inhaled pure ^{14}C-SY aerosols or ^{14}C-SY aerosols in combination with SG (a 0.6 ratio of yellow to green by weight).

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Abstract - continued
Page 2

SY was rapidly cleared from the respiratory tract ($T_{\frac{1}{2}}$ = 3 hr.). SG, however, was retained for a longer time ($T_{\frac{1}{2}}$ > 22 days). The major pathway for excretion of SY metabolites was the feces, but some metabolites were also excreted in urine. There was no effect of SG on the kinetics of distribution and elimination of SY. The rapid absorption, distribution, and excretion of SY compared to SG may explain, in part, the differences in lowest toxic concentration observed in the Phase III toxicity studies conducted at ITRI (>250 mg/m^3 for SY and ≥ 50 mg/m^3 for SG).

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EXECUTIVE SUMMARY

The USAMBRDL has an interest in the potential inhalation toxicity of a yellow dye (SY) and a yellow/green dye mixture (SY/SG) used in colored smoke munitions. The major concern is for the health of the munition production workers who could be exposed to dusts containing the dyes during production of the smoke munitions. The chemical name of the major component of the yellow dye is 2-(2'-quinolinyl)-1,3-indandione (QI) and the major component of the green dye is 1,4-di-p-toluidinoanthraquinone (TA). The SY/SG is a 30:70 mixture of SY:SG.

The Lovelace Inhalation Toxicology Research Institute has conducted a multiphase study of the inhalation toxicity of SY and the SY/SG mixture. Phase I included standardization of methods for generation of aerosols of the test materials and physical/chemical characterization of the aerosols. Phase II consisted of range-finding experiments to determine acute toxic effects from exposure to high concentrations of the dyes and to select exposure concentrations for the next two phases of the study. Phase II also included toxicokinetic studies to determine the disposition of inhaled SY and the effect of combined exposure to SY and SG on the toxicokinetics of SY. In Phase III, four-week exposures of animals to varying concentrations of the dyes were used to determine the lowest exposure concentration that would produce pathological changes. Phase IV consisted of a 90-day subchronic study to determine a no-observable-adverse effects level (NOAEL) of exposure. This is a final report of the work completed in Phase II of these studies.

In the rangefinding studies, no mortalities or life-threatening lesions were observed in rats exposed for as long as 6 hr per day for 5 days to airborne concentrations greater than 1 g/m^3 of either SY or SY/SG. The

particle size of the aerosols employed was about 5 μm mass median aerodynamic diameter (MMAD). Given the predicted respiratory tract deposition pattern of this sized aerosol, the nasal cavities would be expected to receive higher dye concentration than the lung. Even in rats's region of highest predictable dye deposition, only alterations of a minor nature (goblet cell hypertrophy and hyperplasia) were observed in animals from the 5-day repeated dose studies.

These findings indicate that SY and SY/SG have a low order of acute toxicity when inhaled by rats. The information obtained from these short-term studies may be useful in drawing general comparisons with other materials that were tested by equivalent methodologies. However, these data should not be used to suggest that adverse effects are unlikely by other conditions of exposure, such as long-term exposure to the same or lower levels. In the evaluation of health effects resulting from longer term exposure, acute studies can only provide one source of input toward the proper design of a long-term experiment.

In the toxicokinetic studies, rats inhaled pure ^{14}C-SY aerosols or ^{14}C-SY aerosols in combination with unlabeled SG (a 0.6 ratio of yellow to green by weight). Endpoints measured were respiratory function parameters and respiratory tract deposition, lung retention of SG and SY, distribution of ^{14}C-SY equivalents in tissues, metabolism of SY, and pathways for excretion of ^{14}C-SY equivalents.

Respiratory function patterns were not affected by inhalation of either exposure atmosphere. Both total and regional fractional deposition were similar to deposition patterns seen with monodisperse aerosols of similar size. Results indicated that following exposure, SY was rapidly cleared from

the respiratory tract ($T_{1/2}$ ~ 3 hr). SG, however, was retained for a longer time ($T_{1/2}$ > 22 days). Following absorption, SY was extensively metabolized, most likely by the liver and kidney. The major pathway for excretion of SY metabolites was the feces, but some metabolites were also excreted in urine. Very little SY was metabolized to $^{14}CO_2$. By 72 hr after exposure, only 10 percent of the initial inhaled material remained in the body. There was no effect of SG on the kinetics of distribution and elimination of SY. The rapid absorption, distribution, and excretion of SY compared to SG may explain, in part, the differences in lowest toxic concentration observed (> 250 mg/m^3 for SY and < 250 but $\geq$ 50 mg/m^3 for SG) seen in the Phase III toxicity studies conducted at ITRI.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Uses of Laboratory Animals," prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 80-23).

The authors acknowledge the contributions of all members of the staff of the Inhalation Toxicology Research Institute who helped in the completion of this work. The research was supported by the U. S. Army Medical Research and Development Command under a Memorandum of Understanding Agreement No. AT(29-2)-2138/3807 with the Lovelace Inhalation Toxicology Research Institute, which is operated for the U. S. Department of Energy under DOE Contract No. DE-AC04-76EV01013.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

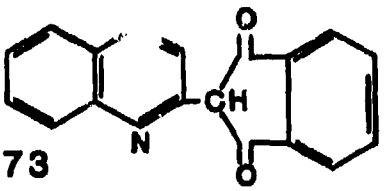
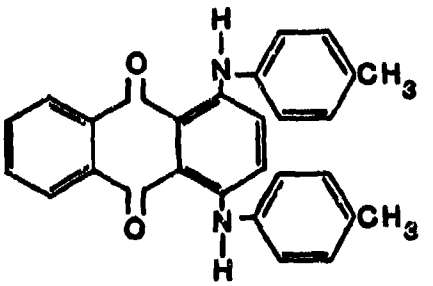
LIST OF FIGURES (Continued)

INTRODUCTION

The U. S. Army needs to obtain information on the inhalation toxicity of the dyes present in colored smoke munitions. The major concern is for the health of munition production workers who could be exposed to fine dusts containing the dyes during production of the smoke munition. In the project, "Studies on the Inhalation Toxicity of Dyes Present in Colored Smoke Munitions", (Project Order #3807) the Lovelace Inhalation Toxicology Research Institute (ITRI) is studying the inhalation toxicity of two dye materials: a yellow dye (SY) and yellow/green dye mix (SY/SG). The chemical name of the yellow dye is 2-(2'-quinolinyl)-1,3-indandione (QI). Synonyms for the dye include C. I. Solvent Yellow 33 and C. I. 47000. If the lot of the dye has been certified by the Federal Drug Administration (FDA) for use in drugs and cosmetics, it is called D & C Yellow No. 11. The green dye (SG) is 1,4-di-p-toluidinoanthraquinone (TA) and has been called C. I. Solvent Green 3, C. I. 61565 or, if FDA certified, D & C Green No. 6. The lots of dye used in these studies were not certified by the FDA for use in drugs and cosmetics. Structures of the two dyes are shown in Figure 1. The SY/SG dye mix contains approximately 30 percent yellow dye and 70 percent green dye. The smoke munitions will contain 42 percent by weight of the dyes. The munition also contains potassium chlorate, magnesium carbonate and sucrose.

The work is being conducted in four phases. Phase I includes standardization of methods for generation of aerosols of the test materials and physical/chemical characterization of the aerosols. Phase II consists of range-finding experiments to determine acute toxic effects from exposure to high concentrations of the dyes and to select exposure concentrations for the next two phases of the study. Phase II also includes toxicokinetic studies to

Figure 1. Chemical structures of solvent yellow and solvent green.

Chemical Name	Structural formula	Common name
2-(2′-quinolinyl)-1,3-indandione Molecular wt - 273		Solvent Yellow
1,4-di-p-toluidinoanthraquinone Molecular wt - 418		Solvent Green

determine the disposition of inhaled SY and the effect of combined exposure to SY and SG on the toxicokinetics of SY. In Phase III, four-week exposures of animals to varying concentrations of the dyes will be used to determine the lowest exposure concentration that will produce pathological changes. Phase IV will be a 90-day subchronic study to determine a no-observable-adverse-effects level (NOAEL) of exposure.

This is a final report of the work completed in Phase II of these studies.

RANGE FINDING STUDIES

INTRODUCTION

The studies in this phase of the project were designed to assess the short-term (acute) inhalation toxicity of SY and a 1:2 mixture of SY and SG referred to as SY/SG mixture. Previous studies indicated that these test materials would have a relatively low acute toxicity since SY had an oral LD50 of > 10 g/kg body weight. The approach used was to begin with inhalation exposures on small numbers of rats to define the range of concentrations that result in 0 to 100 percent mortality. These exposures would be followed by complete LC50 studies, unless few, or no, mortalities were observed in the range finding exposures. This section of the report describes the results of all the acute toxicity inhalation exposures. No mortalities or severe adverse effects were observed in rats exposed for as much as 5 days to concentrations higher than 1 g/m^3 for 6 hours per day. It was deemed unnecessary to conduct LC50 studies.

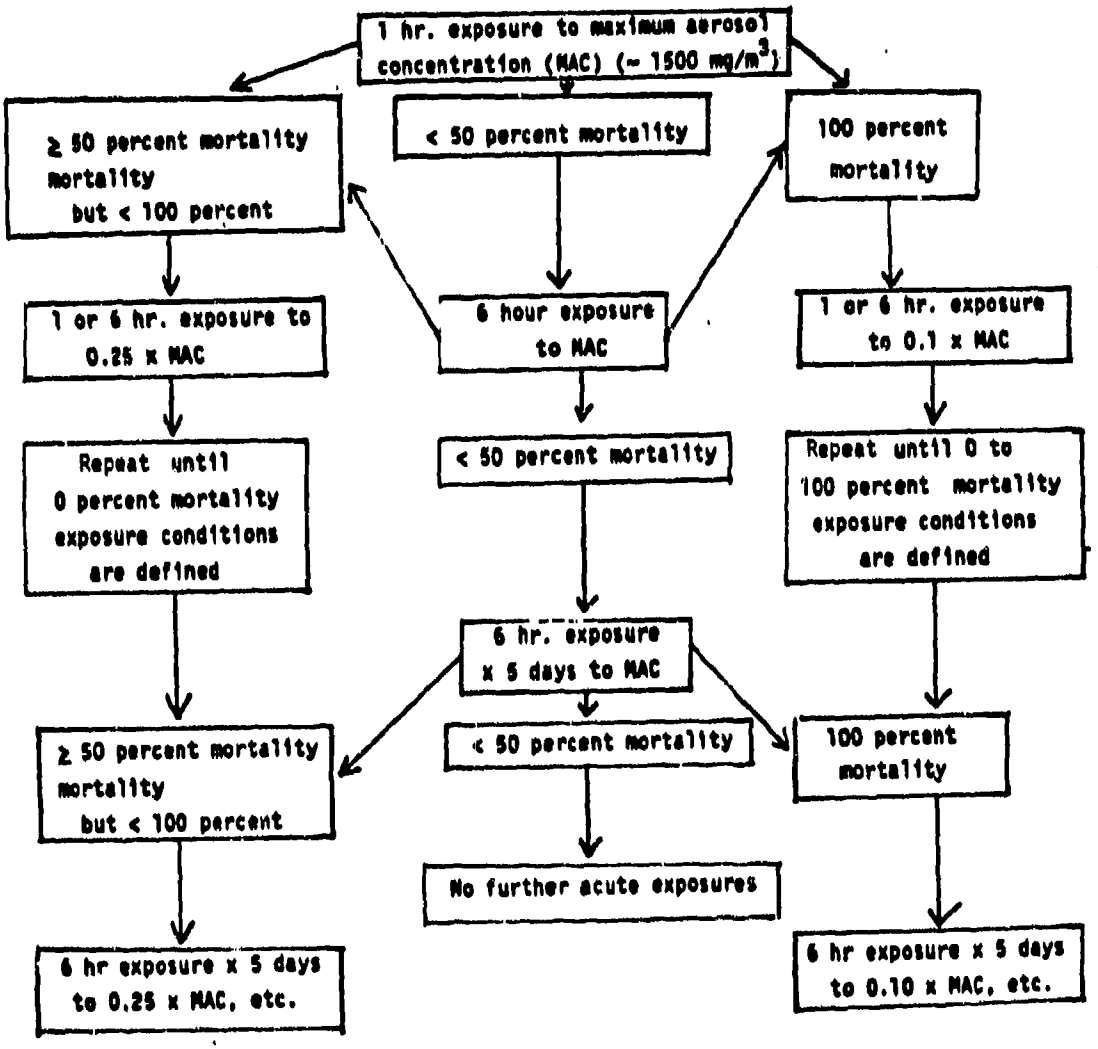
METHODS

Experimental Design

A flow schematic illustrating the design of the range finding exposures is shown in Figure 2. A range finding exposure for 1 hr using a maximum aerosol concentration (MAC) of ~ 1500 mg/m^3 for each test material was to be conducted on three male and three female rats, 14-22 weeks of age. If no animals survived a 14-day observation period, the test material concentration would be lowered by a factor of ten and the experiment repeated with three male and three female rats (Figure 2). If more than 50 percent died at the highest concentration and some survived, the experiment would be repeated at a

Figure 2.

Exposure Study Flow Schematic for Acute Inhalation Toxicity
Range Finding on Aerosolized Dyes



concentration lowered by a factor of four. If less than 50 percent died at the highest concentration, it would be concluded that the one hour LC50 value was greater than this amount and range finding studies using six hours of exposure would be initiated.

For the 6-hr exposures, first a single 6-hour range finding exposure using the MAC exposure level for each test material would be conducted using three male and three female rats, 14-22 weeks of age (Figure 2). If no animals survived a 14-day observation period, the test material concentrations would be lowered by a factor of ten and the experiment repeated. If more than 50 percent died at the MAC level and some survived, the experiment would be repeated at a concentration lowered by a factor of four. If less than 50 percent of the MAC-exposed rats died, it would be concluded that the six-hour LC50 value was greater than this amount and range finding studies using repeated six-hour exposures for five days would be initiated. No pathology was to be conducted on rats used in these range finding studies.

Repeated six-hour exposures for five days were planned only in the event that insufficient (< 50 percent) mortality was observed in the one- and six-hour exposure studies (Figure 2). A range finding exposure using the MAC level of the test material would be conducted on six male and six female rats, 16-20 weeks of age. Animals dying or sacrificed in a moribund condition during the exposure regimen would be subjected to a gross necropsy. Histological examination as described in Table 1 would be conducted. Body weights were to be measured prior to exposure and at 7 and 14 days after completion of the exposure regimen. All surviving animals of each sex would be sacrificed at 14 days after exposure and subjected to a gross necropsy and tissues saved for histology as described in Table 1.

TABLE 1. Organs to be examined grossly, weighed, saved and examined histologically.

Organs	Examine	Weigh	Save Tissues	Routine Histology
Whole Body	X	X		
Skin	X			
Breast	X			
Thymus	X		X	X
Tracheobronchial l.n.	X		X	X
Other l.n.	X			
Spleen	X		X	X
Marrow	X			
Femur	X		X	X
Larynx	X			
Nasal Cavity	X		X	X
Trachea	X			
Lung	X		X	X
Heart	X			
Tongue	X			
Esophagus				
Stomach	X			
Duodenum	X			
Jejunum	X			
Ileum	X			
Cecum	X			
Colon	X			
Liver	X		X	X
Pancreas	X			
Kidney	X		X	X
Urinary Bladder	X			
Epididymus	X			
Testes	X			
Prostate	X			
Uterus	X			
Ovary	X			
Adrenal	X			
Thyroid	X			
Brain	X			
Eye	X			
Lesions	X		X	X

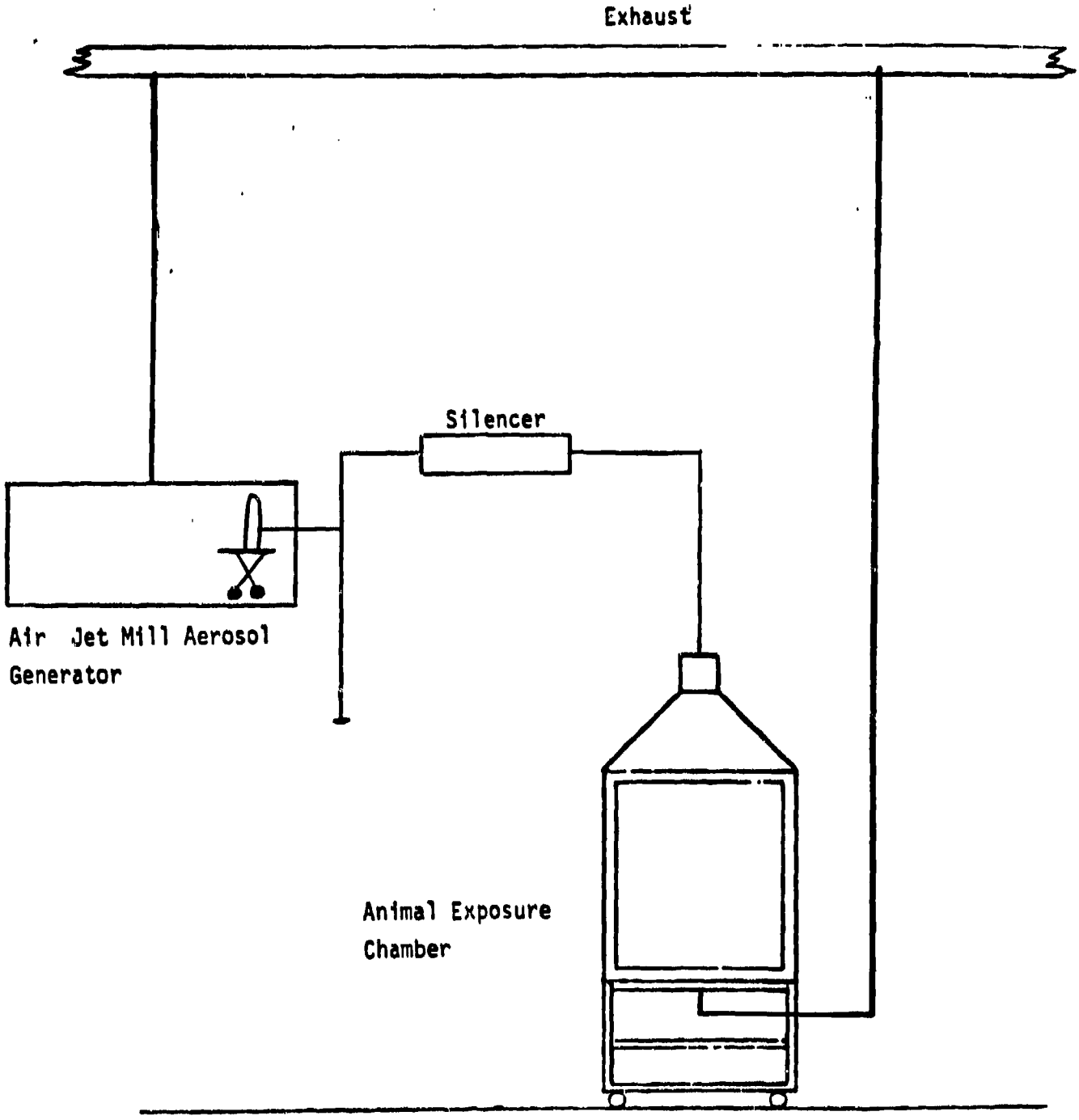
If no animals survived the 14-day observation period, the test material concentrations would be lowered by a factor of ten and the experiment repeated (Figure 2). If more than 50 percent died at the MAC and some survived, the experiment would be repeated at a concentration lowered by a factor of four. If less than 50 percent of the MAC-exposed rats died, it would be concluded that the LC50 value for five daily exposures for 6 hours per day was greater than the MAC and no further acute exposures would be conducted.

Aerosol Generation and Exposure System

A 450 L, Laskin-type animal exposure chamber[1] was used in these range finding studies. A schematic of the exposure system is shown in Figure 3. A Jet-O-Mizer air jet mill (Fluid Energy Processing and Equipment Co., Hatfield, PA) was used to generate aerosols.[2] This generator is a high pressure, opposing air jet system in which material to be aerosolized is continuously fed into the generator using a screw-type dust feeder. The chamber contained a single tier of animal caging and was plumbed with 2-inch stainless steel pipes. The chamber was maintained at a slightly negative pressure (about -0.25 inches water) with an airflow rate of about 400 LPM controlled by an orifice meter calibrated at a pressure difference of 1 inch of water.

The desired particle size was in the range of 2 to 6 μm MMAD with a σ_g of 2.5 or less. These criteria were based on the particle size of the stock dyes and on data showing that this particle size results in some deposition of the aerosol in all areas of the respiratory tract of small rodents.[3] The maximum aerosol concentration was planned to be ~ 1500 mg/m^3. For range finding exposures, aerosol dye concentrations were

Figure 3. Exposure System for Acute Toxicity Range Finding Exposures.



measured by weighing filter samples taken at 15 to 30 min intervals. Aerodynamic size distribution was measured at 30-minute to 3 hr intervals, depending on the exposure regimen, with the Lovelace Multi-Jet cascade impactor.

The exposure chamber was maintained at 26 ± 2°C and 20 to 50 percent relative humidity. The following chamber conditions were monitored and recorded: airflow rate, temperature, humidity and chamber pressure relative to the room. Food and water were not provided during the exposures.

Test Materials and Chemical Analyses

The SY and the SY/SG mixture were supplied by the sponsor. Chemical analyses of the test chemicals to confirm their composition and determine purity were conducted by mass spectrometry and high performance liquid chromatography (HPLC) using a reverse phase column.[4] These same methods of analysis were used to check stability of the dyes under aerosol generation conditions by analyzing filter samples of the aerosols obtained from the exposure chamber during test runs. Both dyes were > 93 percent pure.[4]

Animals

Specific pathogen free, male and female F344/Crl rats, 15-20 weeks of age, were used in these studies. The rats were obtained from the breeding colony at this Institute. All animals were housed two per filter-topped polycarbonate cage on sterilized hardwood chip bedding when not being exposed in the inhalation chamber. Animal rooms were maintained at 20 to 22°C with a 40 to 60 percent relative humidity. The rooms were maintained on a 12-hour light cycle beginning at 6:00 a.m. Food (Lab-Blox, Allied Mills, Chicago, IL)

and water was available ad libitum. Water bottles were changed twice a week. All rodents were changed to clean cages on a weekly basis. Animals were assigned randomly by litter number to various groups, identified with individually numbered ear tags and weighed prior to exposure. Exposures were conducted during the 12-hr light cycle.

RESULTS

The actual maximum aerosol concentrations achieved were between 1000 and 1600 mg/m^3 (Table 2). Initially, six rats (three males, three females) were exposed to the MAC of each test material for 1 hour. This was followed by a single 6 hour exposure of six rats for each test material. The final set of exposures was a 5-day repeated regimen on twelve rats exposed for 6 hours per day. All animals from all experiments were observed for 14 days after completion of the exposures. The rats on the 5-day repeated studies were subjected to a complete necropsy and tissues were saved for histological examination as described in the Methods section. No additional acute exposures were conducted.

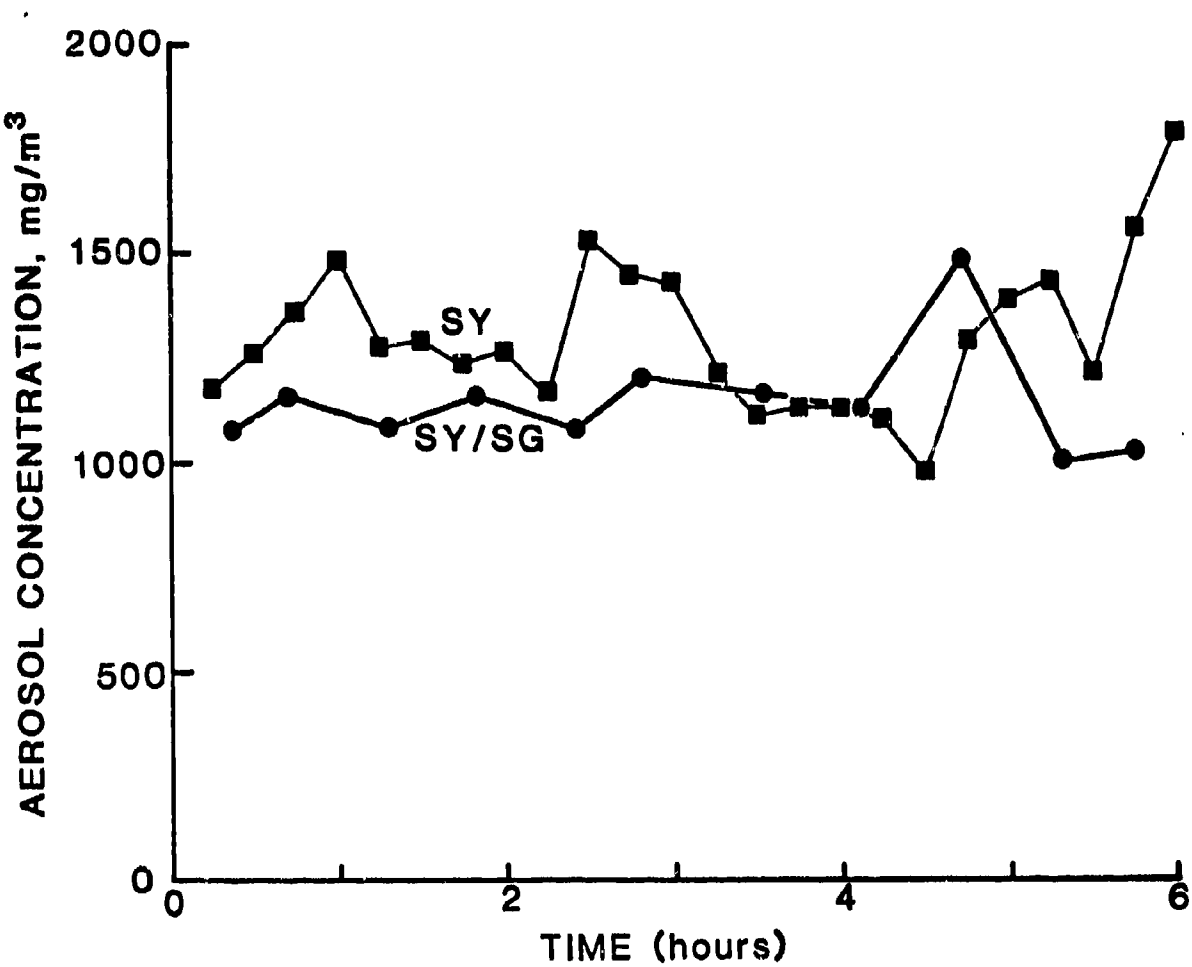
The aerosol characterizations for the three exposures conducted for each test material are given in Table 2. The chamber concentrations ranged from 1000 to 1600 mg/m^3, with a particle size MMAD of 5 to 5.7 μm. An example of the SY and SG/SY mass concentration variations for a typical 6-hr exposure day during the 5-day repeated study is shown in Figure 4.

No mortalities or overt signs of toxicity were observed during any of the exposures. The rats remained inactive during the exposure periods and

TABLE 2. Exposure conditions for the acute inhalation toxicity range-finding exposures.

Test Material	Duration of Exposure (Hr)	Number of Rats	Dye Mass Concentration: Mean ± SE (mg/m^3)	Dye Mass Concentration: Number of Samples	Dye Mass Concentration: Coefficient of Variation (Percent)	Particle Size: MMAD Mean ± SE (μm)
SY	1	3M, 3F	1000 ± 30	20	14	5.1 ± 0.4
	6	3M, 3F	1040 ± 30	51	21	5.7 ± 0.5
	6/day for 5 days	6M, 6F	1290 ± 20	129	20	5.6 ± 0.2
SG/SY	1	3M, 3F	1600 ± 50	24	16	5.0 ± 0.1
	6	3M, 3F	1440 ± 60	24	20	5.5 ± 0.2
	6/day for 5 days	6M, 6F	1490 ± 70	92	44	5.4 ± 0.3

Figure 4. Variation of SY aerosol concentration during a 6-hr exposure.



kept their eyes closed, as would be expected for daylight hours and for such high concentrations of any nuisance dust. No deaths or signs of delayed toxicity were noted 14 days after exposure, making the conduct of LC_{50} studies unnecessary. Loss of 3 to 7 percent of the pre-exposure body weight was noted in all exposure groups within 1 week after exposure (Table 3). However, the rats returned to their pre-exposure body weights by the end of the 14-day observation period.

A gross examination of internal organs was conducted on all rats from the 5-day repeated exposure studies two weeks after exposure. Nasal congestion appeared greater from inhalation of SY than from the SY/SG mixture. Histological examinations were also conducted on some of the tissues subjected to gross observation. The data from repeated exposures to SY are shown in Table 4 and that from the SY/SG mixture in Table 5. The most consistent and striking findings from SY exposure were goblet cell hypertrophy and hyperplasia of the respiratory epithelium in the anterior portion of the nasal cavity. Occasionally, some slight focal chronic inflammation was associated with this lesion. In a few instances, a yellowish brown pigment, presumably dye, was found in macrophages in the submucosa of the respiratory epithelium. Chronic nonsuppurative inflammation of the naso-lacrimal duct was also a consistent finding, as was a serous inflammation of the respiratory epithelium of the naso-volmer organ. These lesions appeared to be compound-related since they were found less frequently and with less severity in unexposed colony rats. The significance of these two findings is problematic. Occasionally foci of acute inflammation and pigment-laden macrophages were seen in the submucosa of the upper trachea. No significant

TABLE 3. Body weights of rats from acute inhalation toxicity range-finding studies.[a]

Exposure Regimen	Number of Rats	SY Exposures Body Weights (g) $\bar{X} \pm SD$ Before Exposure	1 Week After Exposure	2 Weeks After Exposure	Exposure Regimen	Number of Rats	SY/SG Exposures Body Weights (g) $\bar{X} \pm SD$ Before Exposure	1 Week After Exposure	2 Weeks After Exposure
1 hr	6	230 ± 57	220 ± 56	240 ± 58	1 hr	6	220 ± 53	210 ± 53	220 ± 54
6 hr	6	220 ± 51	210 ± 51	220 ± 50	6 hr	6	240 ± 68	230 ± 61	240 ± 64
6 hr/day for 5 days	12	220 ± 73	210 ± 63[b]	240 ± 82	6 hr/day for 5 days	12	240 ± 83	230 ± 82	240 ± 74

[a]The mean daily weight gain of colony animals of the same age was 1.3 ± 0.2 g ($\bar{X} \pm SD$ of 4 determinations made in 6 animals over a 6 week period).

[b]Weight taken on fifth day of exposure.

TABLE 4. Histological observations in tissues from rats exposed to 1.3 g/m3 SY for 6 hr/day for 5 days.

ANIMAL NUMBER	NASAL CAVITY RESPIRATORY EPITHELIUM	NASO-LACRIMAL DUCT	NASO-VOMER ORGAN	TRACHEA	OLFACT. EPI.	LUNG	LIVER
P247	goblet cell hypertrophy and hyperplasia - few macrophages and mast cells in submucosa	chronic nonsuppurative inflammation	serous inflammation in respiratory epithelium	cystic dilation of tracheal glands - pigment macrophage in submucosa	NSL	NSL	NSL
P251	goblet cell hypertrophy and hyperplasia - few macrophages and mast cells in submucosa	chronic nonsuppurative inflammation	serous inflammation in respiratory epithelium	cystic dilation of tracheal glands pigment macrophage in submucosa	NSL	few foci of PMNs and AMs in alveoli	NSL
P252	marked goblet cell hyperplasia	serous inflammation	serous inflammation in respiratory epithelium	NSL	NSL	increased number of AMs	NSL
P253	goblet cell hypertrophy	serous inflammation	not in section	NSL	NSL	NSL	NSL
P254	not available	chronic nonsuppurative inflammation	not in section	a few pigment-laden macrophages in submucosa	NSL	NSL	NSL
P255	goblet cell hypertrophy-serous inflammation on septum	chronic nonsuppurative inflammation	serous inflammation in respiratory epithelium	a few foci of chronic inflammatory cells in submucosa	NSL	NSL	NSL
P267	goblet cell hypertrophy and hyperplasia	chronic nonsuppurative inflammation	serous inflammation in respiratory epithelium	NSL	NSL	NSL	NSL
P268	goblet cell hypertrophy and hyperpl.	chronic nonsuppurative inflammation - severe	serous inflammation in respiratory epithelium	NSL	NSL	NSL	NSL
P269	goblet cell hypertrophy and hyperplasia	chronic nonsuppurative inflammation	serous inflammation in respiratory epithelium	NSL	NSL	NSL	NSL
P270	goblet cell hypertrophy and hyperplasia	chronic nonsuppurative inflammation	serous inflammation in respiratory epithelium	foci of inflammation in upper trachea - some pigment-laden macrophages	NSL	NSL	NSL
P271	goblet cell hypertrophy and hyperplasia - mild	chronic nonsuppurative inflammation	serous inflammation in respiratory epithelium	NSL	NSL	NSL	NSL
P272	goblet cell hypertrophy and hyperplasia - mild	chronic nonsuppurative inflammation	serous inflammation in repiratory epithelium	a few foci of inflammation	NSL	NSL	coagulation necrosis - injection artifact

NSL = no significant lesions
AM = alveolar macrophages
PMN = polymorphonuclear leukocytes

Table 4 (continued)

ANIMAL NUMBER	KIDNEY	SPLEEN	BONE MARROW	THYMUS	LUNG ASSOCIATED L.N. (Some tissues included with thymus)	OTHER
P247	NSL	NSL	NSL	NSL	not in section	None
P251	NSL	NSL	NSL	NSL	not in section	None
P252	NSL	NSL	NSL	NSL	pigment-laden macrophages in sinusoids with mast cells	None
P253	NSL	NSL	NSL	NSL	mast cells in sinusoids few migment-laden macrophages	None
P254	NSL	NSL	NSL	NSL	not available	None
P255	NSL	NSL	NSL	NSL	a few pigment-laden macrophages and mast cells in sinusoids	intestine-congestion
P267	NSL	NSL	NSL	NSL	large amount of pigment in macrophages	ovary-fallopian tube papilloma
P268	NSL	NSL	NSL	NSL	pigments-laden macrophages and mast cells in sinusoids	None
P269	NSL	NSL	NSL	NSL	pigment-laden macrophages and mast cells in sinusoids	None
P270	NSL	NSL	NSL	NSL	not in section congestion	cecum -
P271	NSL	NSL	NSL	NSL	not in section	None
P272	NSL	NSL	NSL	NSL	not in section	None

TABLE 5. Histological observations in tissues from rats exposed to 1.5 g/m^3 SY/SG for 6 hr/day for 5 days

NSL = no significant lesions
NIS = not in section

	NASAL CAVITY			TRACHEA	LUNG (all are congested, CO_2 killed)	LIVER	KIDNEY	SPLEEN	THYMUS	TBLN
Animal Number	Respiratory Epithelium	Nasovolmer Organ	Naselacrimal Duct							
T617	Goblet cell hyperplasia-mod.	Resp.-serous infl. Olfact.-degeneration	Chronic Nonsuppurative infl. slight	NSL	Congestion-focal acute hemorrhage-foci of alveolar histiocytosis	NSL	NSL	NSL	NSL	Pigment-laden macrophages & mast cells
T618	NSL	Resp.-serous infl.	Chronic Nonsuppurative infl. slight	NSL	Foci of alveolar histiocytosis at term airways	NSL	NSL	NSL	NSL	NSL-Medulla not sectioned
T619	Goblet cell hyperplasia-slight	NIS	Chronic Nonsuppurative infl. slight	NSL	NSL	Squamous cyst-1 mm	NSL	NSL	Medullary Hem.	NSL-Medulla not sectioned
T620	Goblet cell hyperplasia-slight	Resp.-serous infl. mild Olfact.-degeneration	Chronic Nonsuppurative infl. slight	NSL	Foci of alveolar histiocytosis at term airways, few	NSL	NSL	NSL	Medullary Hem.	Pigment-laden macrophages
T621	Goblet cell hyperplasia-slight	Resp.-NSL Olfact.-degeneration	Chronic Nonsuppurative infl. slight	NSL	NSL	NSL	NSL	NSL	Medullary Hem.	A few pigment-laden macrophages
T622	Goblet cell hyperplasia-slight	Resp.-serous infl. Olfact.-NSL	Chronic Nonsuppurative infl. slight	NSL	Foci of alveolar histiocytosis at term airways	NSL	NSL	NSL	NSL	NSL-no medulla
T623	Goblet cell hyperplasia-mod.	Resp.-serous infl. Olfact.-degeneration	Chronic Nonsuppurative infl. slight	NSL	Foci of alveolar histiocytosis of term airways	NSL	NSL	NSL	Medullary Hem.	Hemorrhage-acute few pigment-laden macrophages
T624	Goblet cell hyperplasia-mod.	Resp.-serous infl. Olfact-degeneration	Chronic Nonsuppurative infl. slight	NSL	Foci of alveolar histiocytosis at term airways-few	NSL	NSL	NSL	Medullary Hem.	Pigment-laden macrophages
T625	Goblet cell hyperplasia-mod.	Resp.-serous infl. Olfact.-NSL	NIS	NSL-Some pig. macrophages	Foci of alveolar histiocytosis at term airways-very few	NSL	NSL	NSL	NSL	Paracortical hypertrophy
T626	Goblet cell hyperplasia-slight	NIS	Chronic Nonsuppurative infl. slight	NSL	Foci of alveolar histiocytosis at term airways-few	NSL	NSL	NSL	NSL	Pigment-laden macrophages
T627	Goblet cell hyperplasia-severe	Resp.-serous infl. Olfact.-degeneration	Chronic Nonsuppurative infl. slight	NSL	NSL	Few * mononuclear cell infil.	NSL	NSL	Medullary Hem.	NSL
T628	Goblet cell hyperplasia-mod.	Resp.-serous infl. Olfact.-degeneration	Chronic Nonsuppurative infl. slight	NSL	Foci of alveolar histiocytosis of term airways-few	NSL	NSL	NSL	NSL	NSL

*Tissue inadvertently not taken during necropsy

lesions were seen in the lung or olfactory epithelium. Lung-associated lymph nodes frequently had pigment-laden macrophages in the sinusoids.

The most consistent finding from histological examination of tissues from rats exposed to 1500 mg/m^3 of the SY/SG dye for 6 hr/day for 5 days was a goblet cell hyperplasia of the respiratory epithelium in the anterior ventral portion of the nasal cavity. The naso-volmer organ, also in the anterior ventral portion of the nasal cavity, had a mild serous inflammation of the respiratory epithelial portion and degeneration of the olfactory epithelial portion. A slight chronic nonsuppurative inflammation of the epithelium of the nasolacrimal duct was also present. These lesions in the nasal epithelium are probably the result of direct irritation. The significance of the lesions in the naso-volmer organ is not known.

In the lung, many rats had a few focal accumulations of alveolar macrophages that were centered on terminal airways. No lesions were seen in the trachea, although an occasional pigment-laden macrophage could be found in the submucosa. Pigment-laden macrophages were found in the medulla of the tracheobronchial lymph node.

SUMMARY AND CONCLUSIONS

No mortalities or life-threatening lesions were observed in these acute toxicity studies on rats exposed for as long as 6 hr per day for 5 days to airborne dye concentrations greater than 1 g/m^3. The particle size of the aerosols employed was about 5 μm MMAD (Table 2). Given the predicted respiratory tract deposition pattern of this size aerosol, the nasal cavities would be expected to receive a higher dye concentration than the lung. Even in this region of highest predictable dye deposition, only alterations of a minor nature were observed in animals from the 5-day repeated dose studies.

These findings indicate that SY and SY/SG have a low order of acute toxicity when inhaled by rats. The information obtained from these short-term studies may be useful in drawing general comparisons with other materials that were tested by equivalent methodologies. However, these data should not be used to suggest that adverse effects are unlikely by other conditions of exposure, such as long-term exposure to the same or lower levels. In the evaluation of health effects resulting from longer term exposure, acute studies can only provide one source of input toward the proper design of a long-term experiment.

TOXICOKINETIC STUDIES

INTRODUCTION

Limited toxicokinetic studies on the fate of SY in the rat have been reported. Wahlstrom et al.[5] reported that orally administered SY was recovered almost quantitatively in the feces within 48 hours. Approximately 2 percent of the administered dose was in the urine. Intravenous injections of the dye resulted in 60 percent of the dose excreted in the bile at 4 hr and 20 percent excreted in the urine. None of the dye was detected in the blood after 4 hr. In the isolated perfused liver, 70 percent of the administered dye was excreted into bile in 3 hr.

No work has been reported on the toxicokinetics of SG nor on the fate of either dye administered by inhalation. An important aspect of the toxicological evaluation of smoke munition dyes is determination of the deposition and metabolic fate of these dyes following inhalation.

The data obtained in the toxicokinetic studies outlined in this report were used to derive the following information:

1. Pulmonary deposition and retention of inhaled SY.
2. Concentrations of SY in target organs.
3. Major routes of excretion and half-times for excretion of SY.
4. Evaluation of the effect of inhaling a mixture of SG and SY on the disposition of SY.

SYNTHESIS OF ^{14}C-SY

Ring-labeled phthalic anhydride-[phenyl-U-^{14}C] (specific activity 38.6 mCi/mmole) was obtained from Midwest Research Institute (Kansas City, MO). Radiochemical purity was 99 percent as determined by thin layer chromatography. Radiolabeled ^{14}C-2-(2'quinolinyl)-1,3-indandione (SY) was synthesized from the radiolabeled precursor ^{14}C-phthalic acid by the reaction shown in Figure 5. A mixture of 108 mg ^{14}C-phthalic anhydride and 370 µL (2 equivalents) of quinaldine was magnetically stirred and heated in a glycerol bath at 200°C for 1 hr. The resulting dark solid was washed with hexane to remove unreacted quinaldine, dissolved in chloroform, and chromatographed on a silica gel column (22 x 1 cm; 60-200 mesh, Type I; Sigma Chem. Co.) using chloroform as the eluant. This procedure removed dark polymeric material, phthalic acid, and any unreacted phthalic anhydride from the product. A dark reddish/yellow band that eluted after 30 mL contained the solvent yellow. This material was collected, the solvent evaporated, and the reddish residue recrystallized from ethyl acetate, giving 112 mg (56 percent yield). A second crop of crystals from the mother liquors gave another 18 mg, increasing the overall yield to 65 percent. By HPLC analyses (Figure 6), the radiochemical purity of this labeled product was 95 percent.

Figure 5. Synthesis of radiolabeled SY from precursors, quinaldine and phthalic anhydride-[phenyl-U-^{14}C].

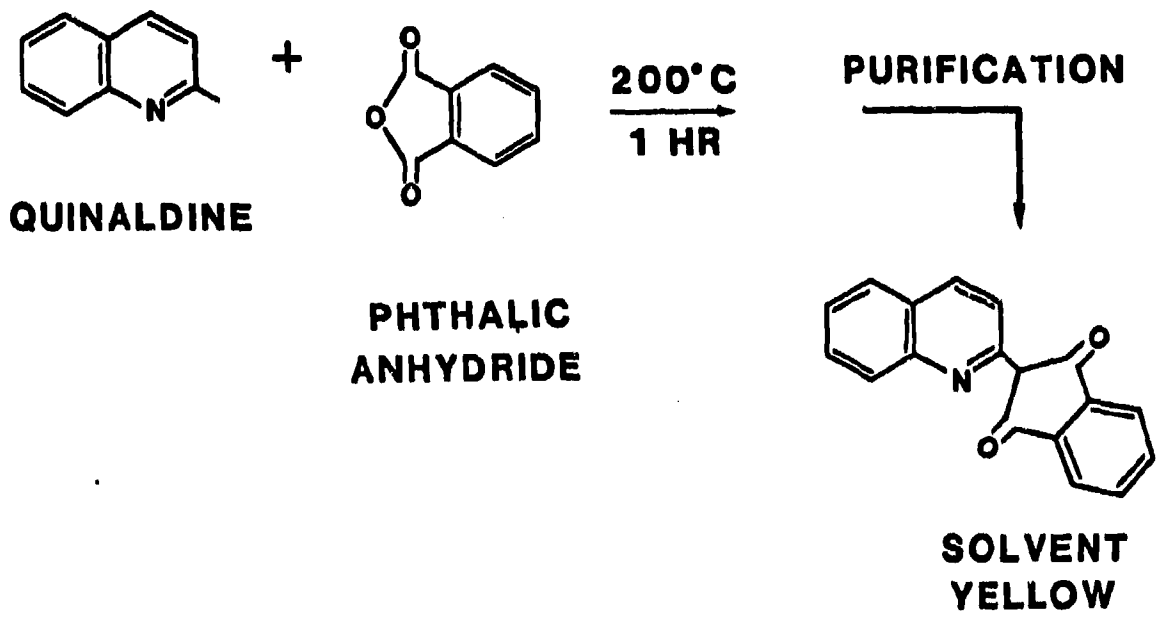
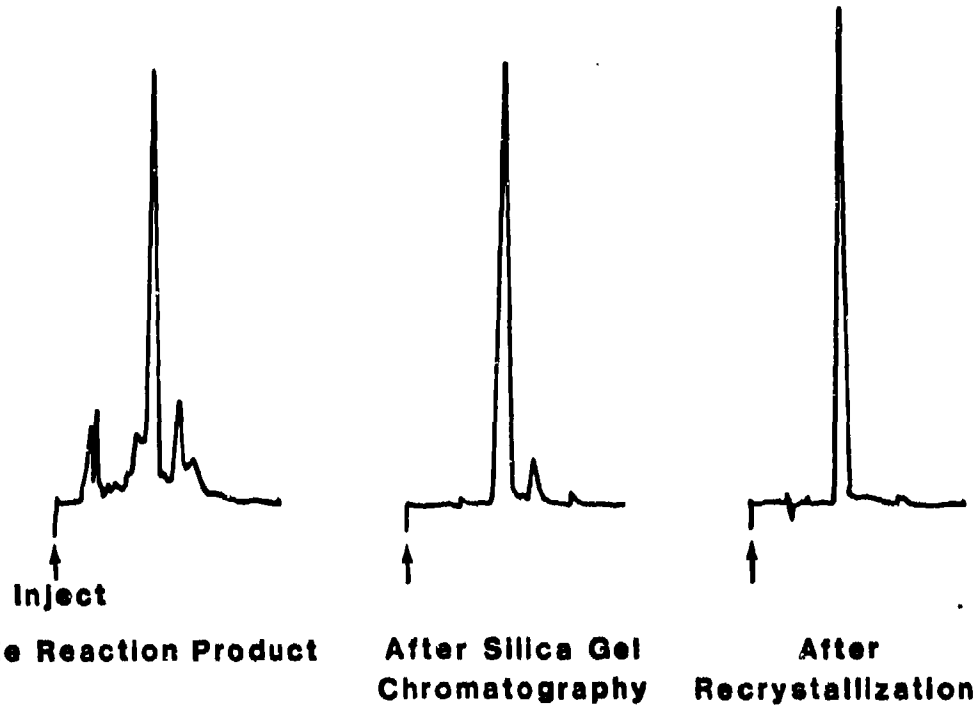


Figure 6. High pressure liquid chromatogram of reaction products from solvent yellow synthesis. HPLC conditions: C18 reverse phase column; 85% methanol in water, 1 mL/min flow rate, UV-visible detector at 435 nm.



GENERATION OF SY AND SY/SG AEROSOLS

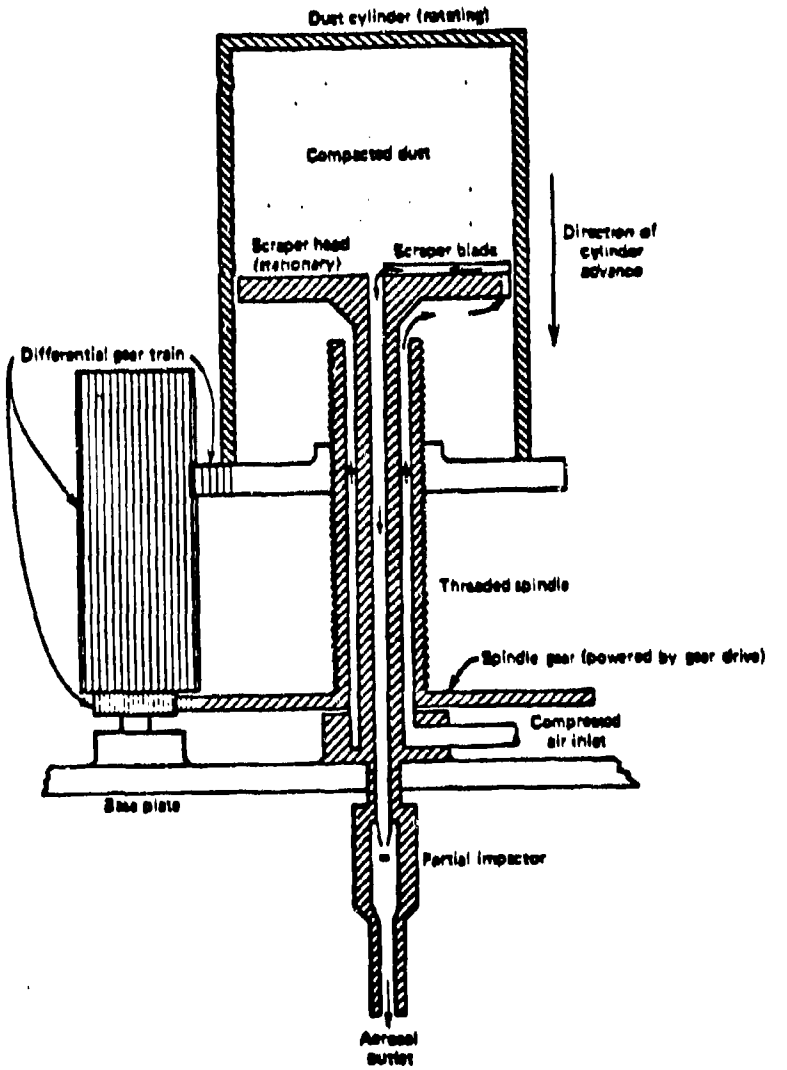
SY Aerosols

Preliminary studies had indicated that SY crystals similar in size to those used in the toxicity studies could be produced by making solutions of 5 mg SY/mL of tetrahydrofuran (THF) containing 1 percent water and precipitating the dye by addition of twice the volume of cold (4°C) hexane. The solution was filtered using a sintered glass filter and allowed to dry under vacuum for 2 hr. Recovery of SY was approximately 67-89 percent. Using this method, 30 mg of ^{14}C-SY (160 µCi/mg) and 210 mg of unlabeled SY were dissolved in THF and precipitated with hexane. The crystals were dried under a vacuum and packed into the generating cup of a Wright Dust Feeder (Figure 7) under 500 lb of pressure.

Aerosols of SY were generated with a gear ratio on the Wright Dust Feeder of 1:1.5. Air flow through the generator was 19 L/min and total flow through the 80 port nose-only exposure chamber was 20 L/min. The duration of the exposure was 60 min, and filter samples (10 min; 1 L/min) for determination of aerosol mass, were taken continuously throughout the exposure. Cascade impactor samples, for determination of the aerodynamic diameter of the particles, were taken (5 min; 1 L/min) during the first and last half of the exposure. At the end of 60 min, air flow through the generator was discontinued.

The average concentration of SY over the 60 min exposure was 43 $\pm$ 6 µg/L (mean $\pm$ SE). The mass median aerodynamic diameter of the particles was 3.4 µm with a geometric standard deviation of 1.7. This aerosol size is comparable to the aerosol in the subacute and subchronic studies, but smaller than the aerosol used in the range finding studies.

Figure 7. Schematic diagram of Wright Dust Feeder. Rotation of the dust cylinder results in the scraper blade removing a certain amount of the compacted dust. Rotation speed controls the amount of dust removed. The dust is then carried by the compressed air out of the cylinder, down the spindle, and into the exposure chamber.



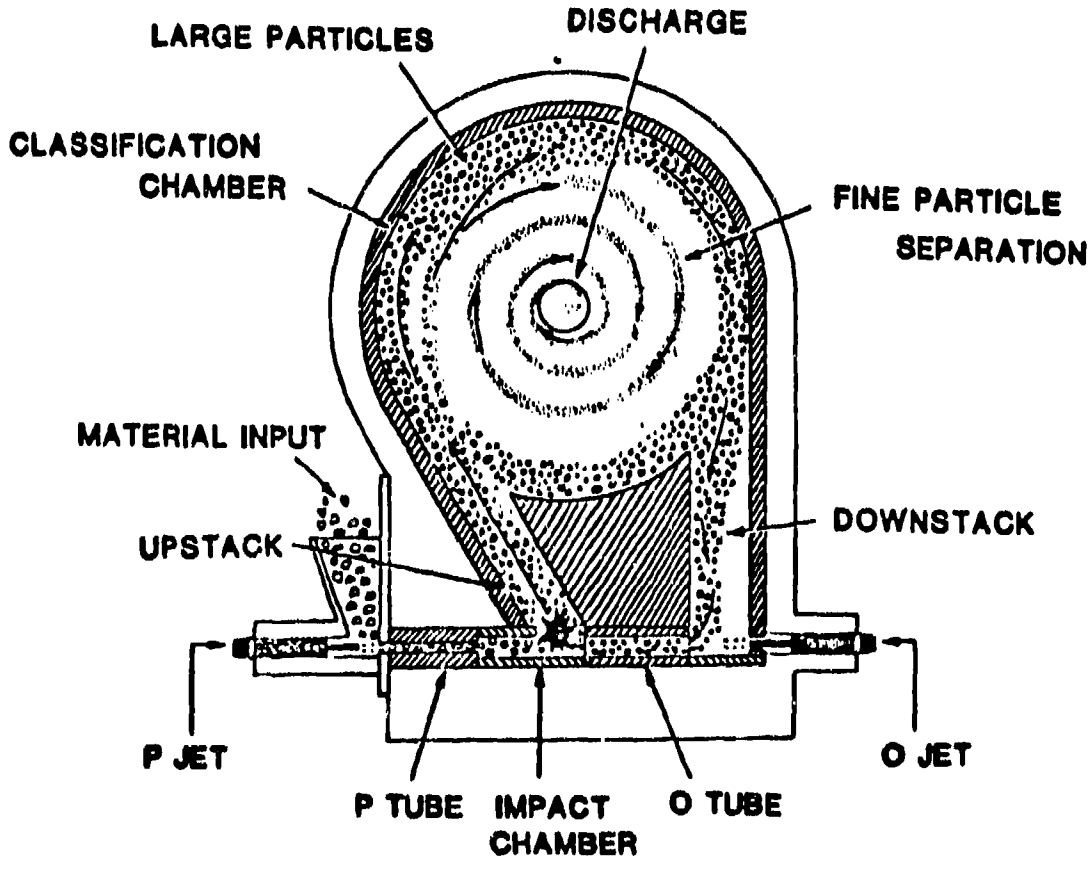
SY/SG Aerosols

The ^{14}C-SY had to be homogenously mixed with the unlabeled SY and SG. A method was developed by which the SG and the ^{14}C-SY could be dissolved and precipitated keeping the ratio of the two dye components constant. SG and tracer amounts of ^{14}C-SY were dissolved in THF. The ^{14}C-SY/SG was precipitated with a 20:1 ratio of deionized water to THF. The weight of the dried, precipitated dye indicated that total recovery by weight was 91 percent. Recovery of ^{14}C was 97 percent, indicating that the SY and SG were precipitated out at the same ratio in which they were present in the original mixture. For the inhalation exposures, the ^{14}C-SY was incorporated into the yellow/green mix with a specific activity of 5.4 uCi/umole SY, and a yellow/total dye ratio of 0.38. This compares to a ratio of 0.30 in the stock dye.

The SY/SG aerosol was generated using a modified Trost-Jet Mill (Figure 8). The air pressure on the inlet jet was 55 psi and the air pressure on the high pressure side was 80 psi. Flow through the 80 port nose-only exposure system was 10 L/min. The precipitated dye was fed into the mill manually. The duration of the exposure was 60 min, and filter samples were taken continuously throughout the exposure (5 min; 1 L/min). Cascade impactor samples were taken (5 min; 1 L/min) once during the first and once during the last half of the exposure.

The average concentration, determined by weighing filter samples collected during the exposure, was 246 µg/L $\pm$ 16 µg/L. (mean $\pm$ SEM). Radiochemical analysis of solutions of the aerosol collected on the filters indicated that the concentration of SY generated was 93 µg/L. By difference the concentration of the SG was 154 µg/L. The particle size was 2.6 µm mass median aerodynamic diameter, with a geometric standard deviation of 1.7.

Figure 8. Schematic diagram of the modified Trost-Jet-Mill. Two directly opposing streams of air impact dye particles resulting in deaglomeration of the particles. The particles move on to a classification chamber, with the smallest particles moving to the inside and finally out through a discharge port. The discharge port is connected to the exposure chamber. The larger particles continue to circulate, impact again, until they exit as smaller particles. Suction is created at a feed port by the passage of air through the air inlet chamber below it. The impaction is created by a 30% pressure differential between the O (opposing) and P (pusher) jets.



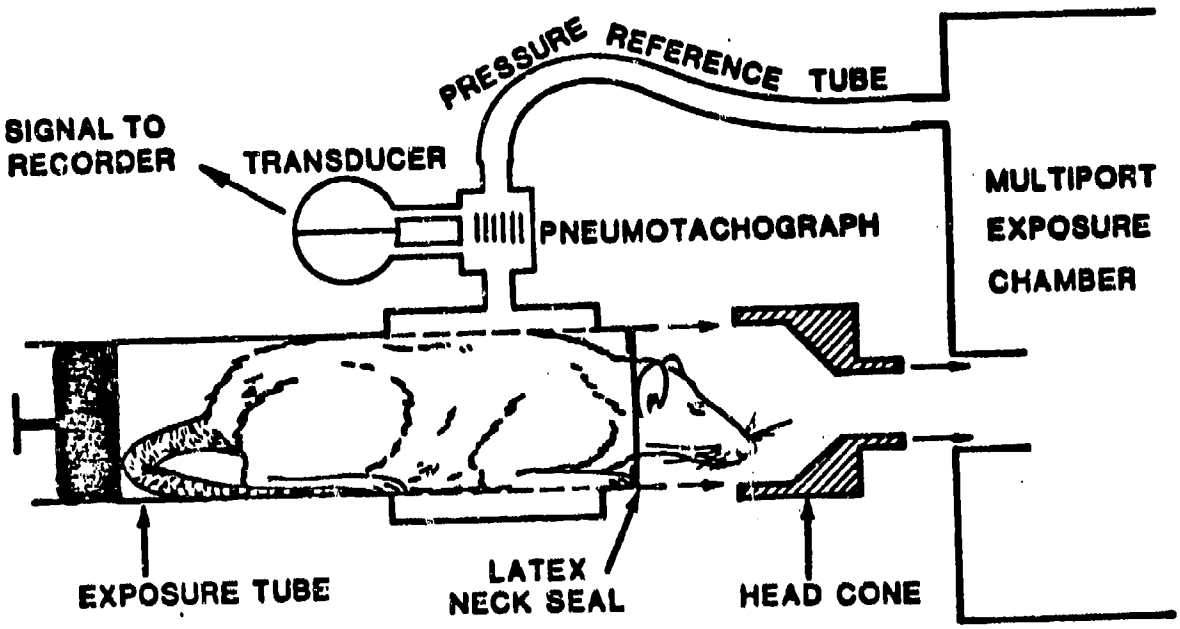
ANIMAL EXPOSURES

Male F344/N rats used in these exposures were approximately 20 weeks of age. They were divided into three groups. The first group of 5 rats was exposed in plethysmographic tubes to monitor respiratory patterns during the exposure. A second group of 6 rats was exposed in conventional tubes and placed in glass metabolism cages at the end of exposure for collection of urine, feces, and expired CO_2 for 70 hr after exposure. The third group of 27 rats was sacrificed 3 per group at predetermined times after exposure, and selected tissues were taken to be analyzed for ^{14}C. Treatment of each of these three groups is described in detail below.

Respiratory Tract Absorption

The plethysmographic exposure unit is shown in Figure 9. The unit was a modified exposure tube with a removable front portion to provide access to the rat's head and an outer sleeve for the rat's torso. A latex collar (Hygiene Corp., Akron, OH) fit snugly around the rat's neck, physically isolating the head chamber from the body chamber, and allowed for measurement of air movements caused by volume changes resulting from the rat's breathing. The neck of the rat was shaved on the day before exposure, at which time body weights of all test rats were determined. The exposure tube wall was perforated in the area of the rat's thorax and communicated with an outer sleeve to which a pneumotachograph (Fleisch size 0000; OEM Medical; Richmond, VA) was attached. Because the exposure tube had a negative pressure relative to that of its surroundings (glove box), the reference side of the pneumotachograph was vented to the exposure chamber to equalize pressure between the plethysmograph and the exposure chamber. Flow through the

Figure 9. Schematic of plethysmograph used for respiratory measurements of rats.



pneumotachograph was measured using a differential pressure transducer (MP45/6-14, Validyne, Northride, CA), a preamplifier, and a Data Logger Computer (Model DL-12; Buxco Electronics, Inc., Sharon, CT). Digitalized and averaged values for frequency and minute volume were displayed on an on-line terminal. Breath-by-breath total volume traces were displayed on a strip-chart recorder (Visicorder, Model 960C, Honeywell, Denver, CO). The volume, linearity, and frequency response of the pneumotachographs and pressure transducers were tested using syringes and a respirator pump; they were found to be accurate to within 2 percent in the ranges required for this study.

The amount of ^{14}C deposited in the respiratory tract during the 1-hr exposure was determined as follows. Within 2 min after the end of each 1-hr exposure, the rats maintained in the plethysmographic exposure units were sacrificed by an IP injection of 1 mL of euthanasia solution (T61; American Hoechst Corp., Somerville, NJ) and depelted. The entire depelted carcass was digested in 1 vol (1 mL of solution per g of carcass) of 20 percent tetraethyl ammonium hydroxide (TEAH) in water at room temperature. After digestion, triplicate 500 mg samples of the digest were weighed, and 1 mL of concentrated HCl (to neutralize) and 0.5 mL of 30 percent hydrogen peroxide (to decolorize) were added to the vials. After the addition of scintillation fluid (Ready-Solv, EP), samples were analyzed for radioactivity in a liquid scintillation spectrometer. Quench correction was by automatic external standard, with sufficient counts accumulated to give < 10 percent error with a 95 percent confidence interval.

Table 6 summarizes the respiratory measurements and fractional deposition of ^{14}C in rats exposed to either SY alone or to a combination of

TABLE 6. Respiratory measurements and fractional deposition of SY in rats exposed to SY alone or to a combination of SY and SG dyes.[a]

Exposure	Aerosol Concentration (μg/L)	Frequency (breaths/min)	Tidal[b] Volume (mL/breath)	Minute Volume mL/min	Volume Inhaled (L)	SY Inhaled (nmoles)	SY Deposited[c] (nmoles)	Fractional Deposition[d] (%)
SY	43 μg SY	100 ± 12	1.7 ± 0.1	170 ± 12	10 ± 0.8	1580 ± 40[e]	660 ± 140	41 ± 6
SY/SG	93 μg SY + 154 μg SG	83 ± 17	1.9 ± 0.2	160 ± 20	9.6 ± 1.3	3180 ± 40[f]	850 ± 270	25 ± 6

[a]Values are $\bar{X}$ ± SE.

[b]Volumes are corrected for saturation with water vapor at body temperature.

[c]Based on ^{14}C detected in depelted carcass of rats exposed in plethysmograph tubes and sacrificed immediately after the exposure.

[d]Fractional deposition was the ratio of SY deposited to SY inhaled x 100.

[e-f]Parameters with different letters are significantly different from each other at $p < 0.05$ by a one way analysis of variance.

SY and SG. A one way analysis of variance indicated that, at a significance level of 0.05, there was insufficient evidence to reject the null hypothesis that the samples come from populations with equal means for breathing frequency, tidal volume or minute volume of rats exposed to either aerosol. The concentration of SY in the SY/SG exposure was twice that of the pure SY exposure, but this was not reflected in the amount of SY deposited in the rats.

Due to both the variability in the amount deposited and an apparent trend toward a lower fractional deposition of the SY/SG aerosol, there was no significant difference in the amount of SY deposited in rats inhaling either SY alone (660 $\pm$ 140 nmoles) or the SY/SG mix (850 $\pm$ 270 nmoles solvent yellow). The trend toward lower fractional deposition for the SY/SG aerosol may have been a result of the smaller particle size in the SY/SG dye exposures (2.6 μm) compared to the the aerosols generated with SY alone (3.4 μm). The increased deposition for the larger 3.4 μm particle was probably the result of increased upper respiratory tract deposition of the larger particle. Similar results have been reported for monodisperse particles of 2-3 μm.[6]

Tissue Distribution

At the end of exposure, some rats were housed individually in polycarbonate cages with hardwood chip bedding and supplied with food and water. At predetermined times after exposure to either SY alone or SY combined with SG, 3 rats per time point were sacrificed by an IP injection of T61 euthanasia solution, and the following tissues were removed and weighed: blood sample, liver, brain, thyroid, thymus, heart, lung, trachea with larynx, turbinates, spleen, kidneys, muscle sample, bone (femur), fat sample

(perirenal and subcutaneous), skin sample (ears), adrenal glands, testes, urinary bladder, stomach, small intestine sample, and large intestine sample. The last 4 tissues were emptied of contents.

Duplicate 250 mg samples (or for small samples, the whole tissue) of the above tissues were weighed and allowed to dry at room temperature for subsequent oxidizing to water and $^{14}CO_2$ in a tissue oxidizer (Packard, Model 306B; Downers Grove, CA). Radioactive CO_2 produced was quantitated in a liquid scintillation spectrometer as described above.

Data obtained for each tissue sample were expressed as nmoles of ^{14}C-SY equivalents per gram of tissue in rats exposed to SY alone or to SY combined with SG. For tissues containing the highest concentrations of ^{14}C (lung, liver, kidney, stomach, spleen, and blood; Figure 10 A-F), a non-linear function, was fit to the data, expressed as concentration of ^{14}C in tissues as a function of time, by non-linear least squares regression analysis using a pseudo-Gauss-Newton algorithm (BMDP, University of California, 1979).

$$F(t) = Ae^{-kt} + B,$$

where F(t) was the fraction yet to be eliminated,
A and B were nmoles ^{14}C/g tissue, and
k (in units of hr^{-1}) was the apparent rate constant for elimination.
Half-times of elimination of ^{14}C were determined from the equation:

$$T_{1/2} = 0.693/k,$$

where k (in units of hr^{-1}) was the apparent rate constant for elimination obtained from regression analysis. Rate constants and half-times of elimination are shown in Table 7.

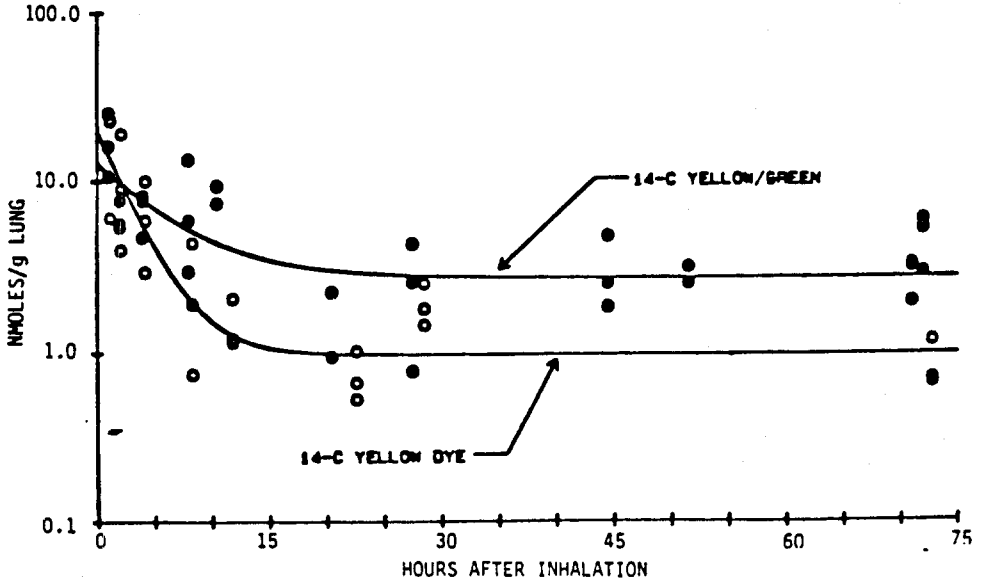
The tissue distribution data were expressed as nmoles of ^{14}C-SY equivalents per tissue at 1 hr after each exposure (Table 8). The data were also expressed as percent of the nmoles of SY deposited in the entire respiratory tract. At 1 hr after exposure, approximately 30 to 50 percent of the initial deposited amount could be found in the major organs and large tissue masses such as blood, intestines, muscle, bone, skin, and fat. The largest amounts were found in the liver, lung, fat, muscle, and skin. The remainder was probably associated with the contents of the gastrointestinal tract, most likely translocated there through mucociliary clearance from the upper respiratory tract. This information and the tissue distribution data in Figure 10 and Table 7 suggested that most of the SY that was inhaled and deposited was rapidly cleared, absorbed into the blood and distributed to other tissues. Only a small fraction of the total amount deposited can be found in any tissue.

Lung Retention of Green Dye

Chemical analyses of dye in the lungs of rats exposed to a mixture of SY and SG indicated that SG was retained in the lungs to a greater extent than SY. To determine if this were the case, the remaining lung samples obtained from rats exposed to ^{14}C-SY in combination with SG were homogenized and chemically extracted to remove the SG. Lung tissue was homogenized in 1 mL of deionized water. The homogenate was transferred to a centrifuge tube, and was extracted three times with 2 volumes of acetonitrile (ACN). The acetonitrile extracts were pooled and evaporated to dryness at room temperature under a

Figure 10. Concentration of ^{14}C-SY equivalents in (A) lung, (B) liver, (C) kidney, (D) stomach, (E) spleen and (F) blood with time after exposure to SY aerosols alone or in combination with SG. Filled circles are data obtained from exposures to SY combined with SG. Open circles are data obtained from exposures to SY alone. Lines are best fits to the data by non-linear regression analysis. Components and rate constants associated with these fits can be found in Table 7.

A. LUNG



B. LIVER

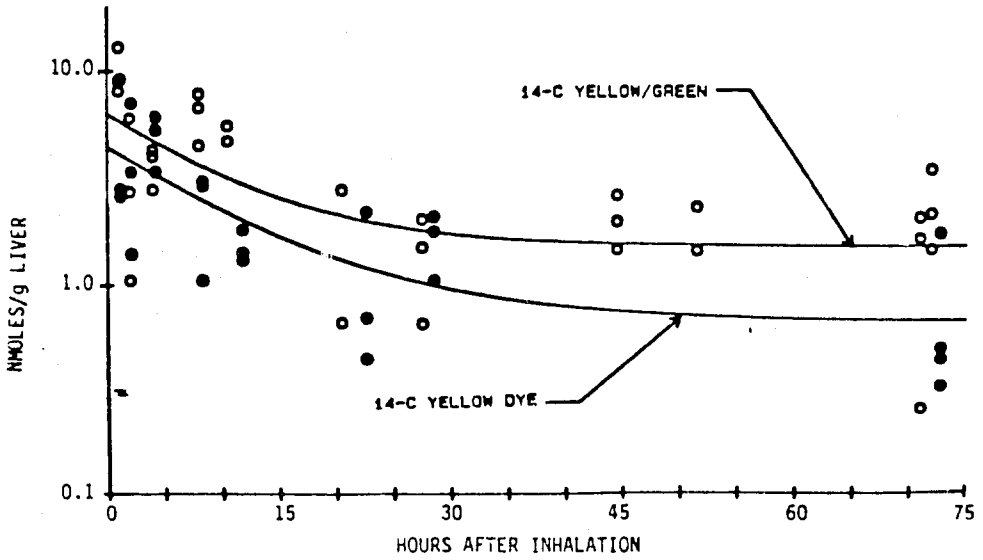
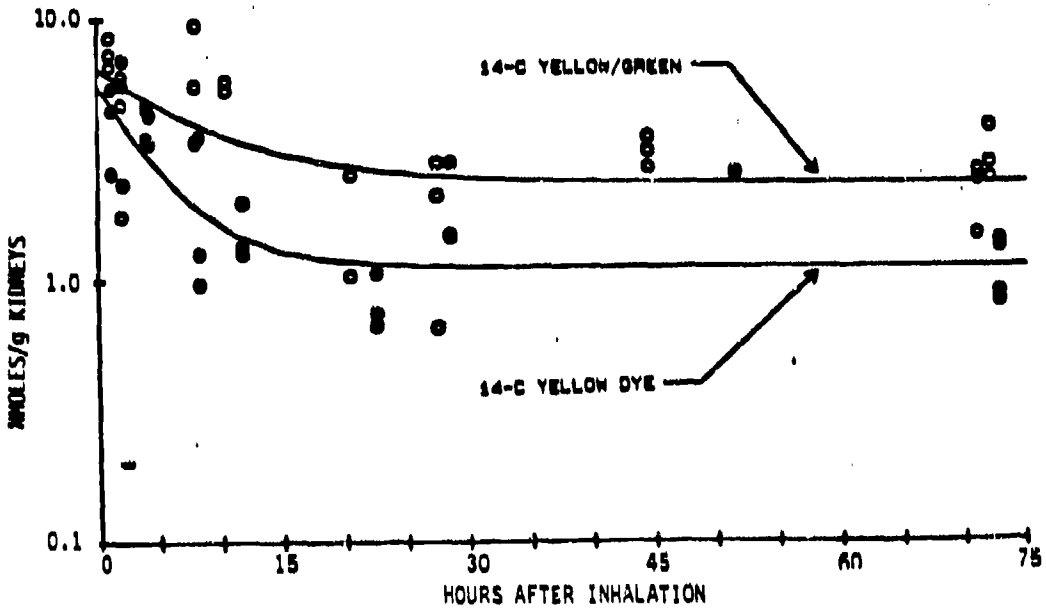


Figure 10.

C. KIDNEY



D. STOMACH

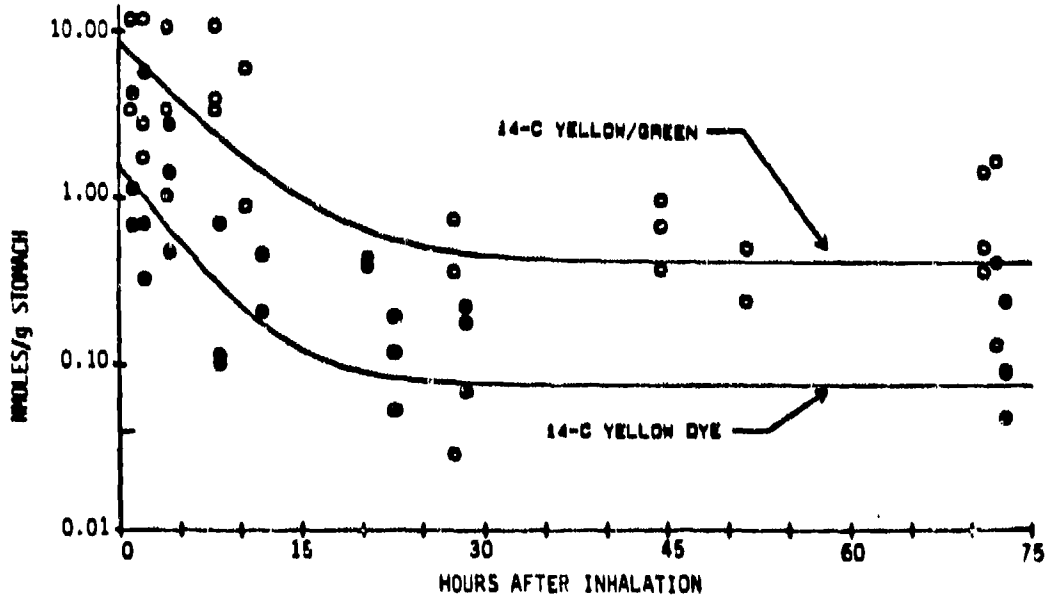
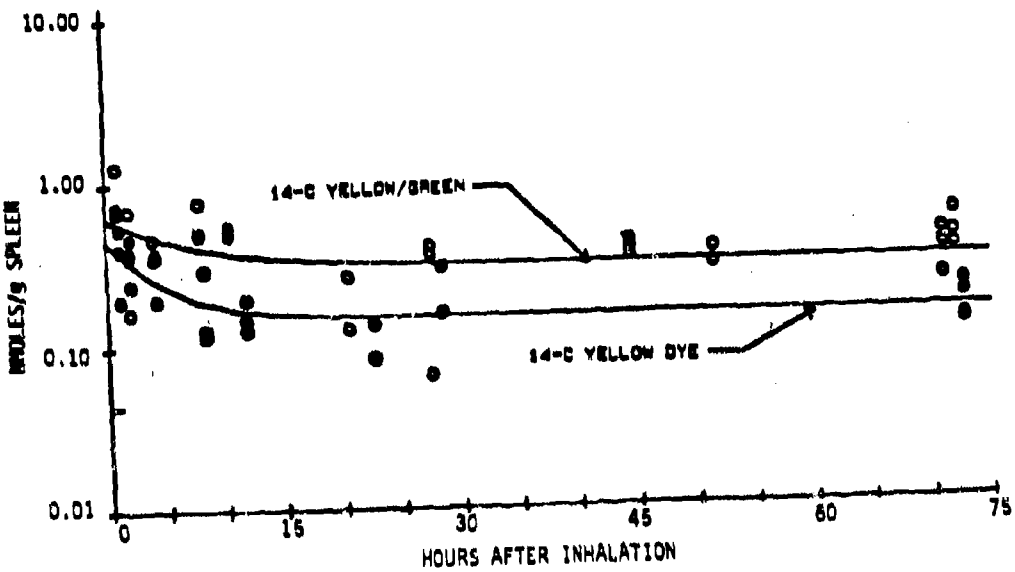


Figure 10.

E. SPLEEN



F. BLOOD

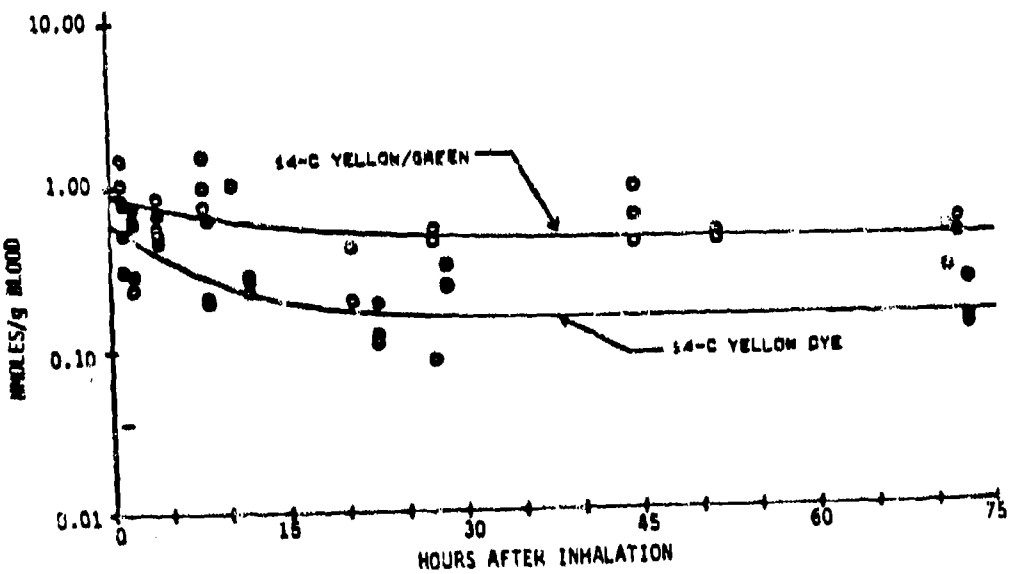


TABLE 7. Apparent rate constants and half-times for elimination of ^{14}C-SY equivalents from tissues after exposure to SY aerosols alone or in combination with SG.[a]

		Short-Term[b]			Long-Term[b]		
Tissue	Exposure[c]	Component A (nmoles/g)	Rate Constant (hr^{-1})	$T_{1/2}$[d] (hr)	Component B (nmoles/g)	Fraction[e]	N
Lung	SY	19 ± 7	0.35 ± 0.09	2	1.0 ± 0.2	0.05	25
	SY/SG	10 ± 4	0.17 ± 0.08	4	2.7 ± 0.3	0.21	30
Liver	SY	4 ± 0.8	0.09 ± 0.03	8	0.67 ± 0.22	0.15	25
	SY/SG	5 ± 2	0.10 ± 0.06	7	1.5 ± 0.3	0.24	30
Kidney	SY	4 ± 1	0.22 ± 0.08	3	1.1 ± 0.2	0.20	25
	SY/SG	4 ± 1	0.12 ± 0.08	6	2.3 ± 0.3	0.36	30
Stomach	SY	1 ± 0.8	0.23 ± 0.09	3	0.08 ± 0.03	0.05	25
	SY/SG	8 ± 3	0.18 ± 0.07	4	0.4 ± 0.1	0.05	30
Blood	SY	0.5 ± 0.2	0.15 ± 0.06	5	0.15 ± 0.03	0.25	25
	SY/SG	0.5 ± 0.2	0.09 ± 0.11	8	0.45 ± 0.07	0.50	30
Spleen	SY	0.3 ± 0.2	0.25 ± 0.13	3	0.15 ± 0.02	0.33	25
	SY/SG	0.3 ± 0.2	0.15 ± 0.17	5	0.31 ± 0.03	0.50	30

[a]Data expressed as parameter estimate ± SD.

[b]Short and long term components determined from a non-linear least squares regression analysis of the equation:

$$F(x) = Ae^{-Kt} + B$$

where F(x) is nmole ^{14}C-solvent yellow equivalents/g tissue with time after exposure,
A is the short term component,
K is the elimination rate constant in hr^{-1} associated with that component, and
B is the long term component. The elimination rate constant for B cannot be accurately determined because no long-term sacrifices of animals were made.

[c]Tissues taken from rats exposed to ^{14}C-solvent yellow alone (SY) or solvent yellow in combination with solvent green.

[d]The half-time in hr was calculated from the equation $T_{1/2} = \ln 2/k$ where k is the elimination rate constant described in the above equation.

[e]The fraction associated with the long-term component was calculated as follows,

$$\text{fraction} = \frac{B}{A+B}$$

where A and B are as defined above.

TABLE 3. Nanomoles of ^{14}C-SY equivalents in tissues at 1 hour after exposure to SY aerosols alone or in combination with SG. (Data expressed as nmoles ^{14}C per tissue)

Tissue	SY Alone ^{14}C-SY Equivalents	Percent of Initial Respiratory Tract Burden	SY Combined with SG ^{14}C-SY Equivalents	Percent of Initial Respiratory Tract Burden
ADRENAL	0.08 ± 0.04[a]	0.01	0.26 ± 0.05	0.03
BRAIN	0.69 ± 0.12	0.10	2.3 ± 0.08	0.27
HEART	0.59 ± 0.22	0.09	0.93 ± 0.33	0.11
KIDNEY	9.0 ± 1.8	1.4	15 ± 0.65	1.8
LARYNX/TRACHEA	0.90 ± 0.20	0.14	4.3 ± 1.3	0.51
LIVER	58 ± 28	8.8	93 ± 12	11
LUNG	21 ± 6.7	3.2	19 ± 1.2	2
LYMPH NODES	0.02 ± 0.003	0.003	0.03 ± 0.01	0.004
SPLEEN	0.26 ± 0.07	0.004	0.45 ± 0.08	0.05
STOMACH[b]	3.1 ± 1.7	0.5	11 ± 3.5	1.3
TESTES	1.3 ± 0.4	2.0	4 ± 0.69	0.5
THYMUS	0.19 ± 0.08	0.03	0.29 ± 0.10	0.03
THYROID	0.04 ± 0.02	0.01	0.03 ± 0.01	0.004
TURBINATES	11 ± 9	1.7	7.5 ± 3.9	0.88
URINARY BLADDER	0.36 ± 0.02	0.05	0.14 ± 0.07	0.02
BLOOD[c]	6.5 ± 1.1	1.0	14 ± 2	1.6
BONE (FEMUR)[c]	4.7 ± 1.4	0.71	76 ± 2	8.9
SKIN (EAR)[c]	41 ± 9.2	6.2	110 ± 40	13
FAT[c]	20 ± 4.0	3.0	34 ± 0.3	4
INTESTINES[b,c]	3.9 ± 1.5	0.6	17 ± 8	2
MUSCLE[c]	24 ± 7.7	3.7	50 ± 10	6

[a] Data expressed as mean nmoles ^{14}C-SY equivalents/total tissue ± SE.

[b] Emptied of contents.

[c] Data for tissue estimated using published values for tissue weights.[7]

stream of N_2. One 1 mL of ACN was added to the dried extract containing the SG, and allowed to sit for 48 hr to bring the SG into solution.

The SG was quantitated by injecting 50 µL of the ACN solution onto an analytical high pressure liquid chromatography column (RP-C18). The SG was separated using an isocratic elution system of 95 percent ACN/5 percent H_2O. The flow rate was 1 mL/min. The absorbance of the eluant at 280 nm was recorded on a strip chart recorder. Standards of pure SG were also chromatographed. Peak height of the lung samples was compared to that of the standards to quantitate the amount of SG remaining in the lungs.

The retention of SG in the lungs of rats exposed to ^{14}C-SY combined with SG is shown in Figure 11. A single component, negative-exponential function, fit to the data by non-linear regression analysis, indicated that the SG did not clear the lungs to any substantial extent during the 70 hr after exposure. A minimum estimate of the half-time for clearance was approximately 22 days, but analysis of SG in lung tissue at longer times after exposure would be required to determine this half-time accurately.

The function describing SG retention indicated that 115 nmoles of SG was deposited in the lungs and bronchi during the exposure to the SY/SG aerosol. Since SY and SG would be expected to deposit in the same ratio as they were found in the aerosol generated, one can calculate the amount of SY deposited in the lungs and bronchi, compared to the total respiratory tract. These data, presented in Table 9, suggest that 14 percent of the total respiratory tract deposition of SY was in the lungs and bronchi. This value compares favorably to that of 22 percent determined by Raabe et al. (1971) for 3.05 µm monodisperse particles.

Figure 11. Retention of SG in lungs of rats expoded to a combination of ^{14}C-SY and SG.

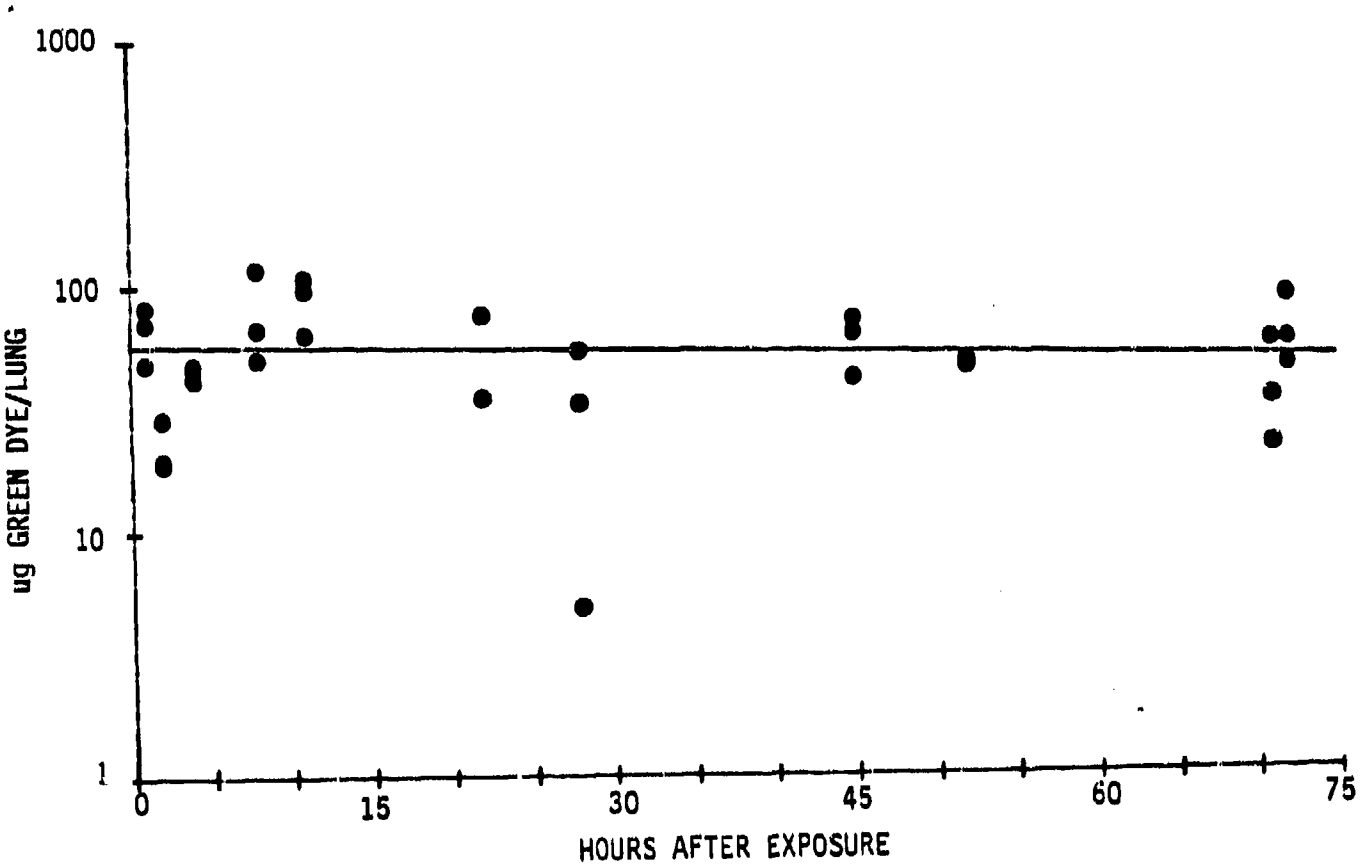


TABLE 9. Regional deposition of ^{14}C-SY and SG following exposure to 2.6 um aerosol particles of SY and SG.

Composition of Exposure Atmosphere	Total Deposition µg (nmoles)	Deposition in Upper Respiratory Tract µg (nmoles)	Deposition in Lungs and Bronchi µg (nmoles)	Percent[a]
93 µg SY/L	230 (850)	200 (730)	32 (110)	14
154 µg SG/L	360 (860)	310 (740)	50 (115)	14

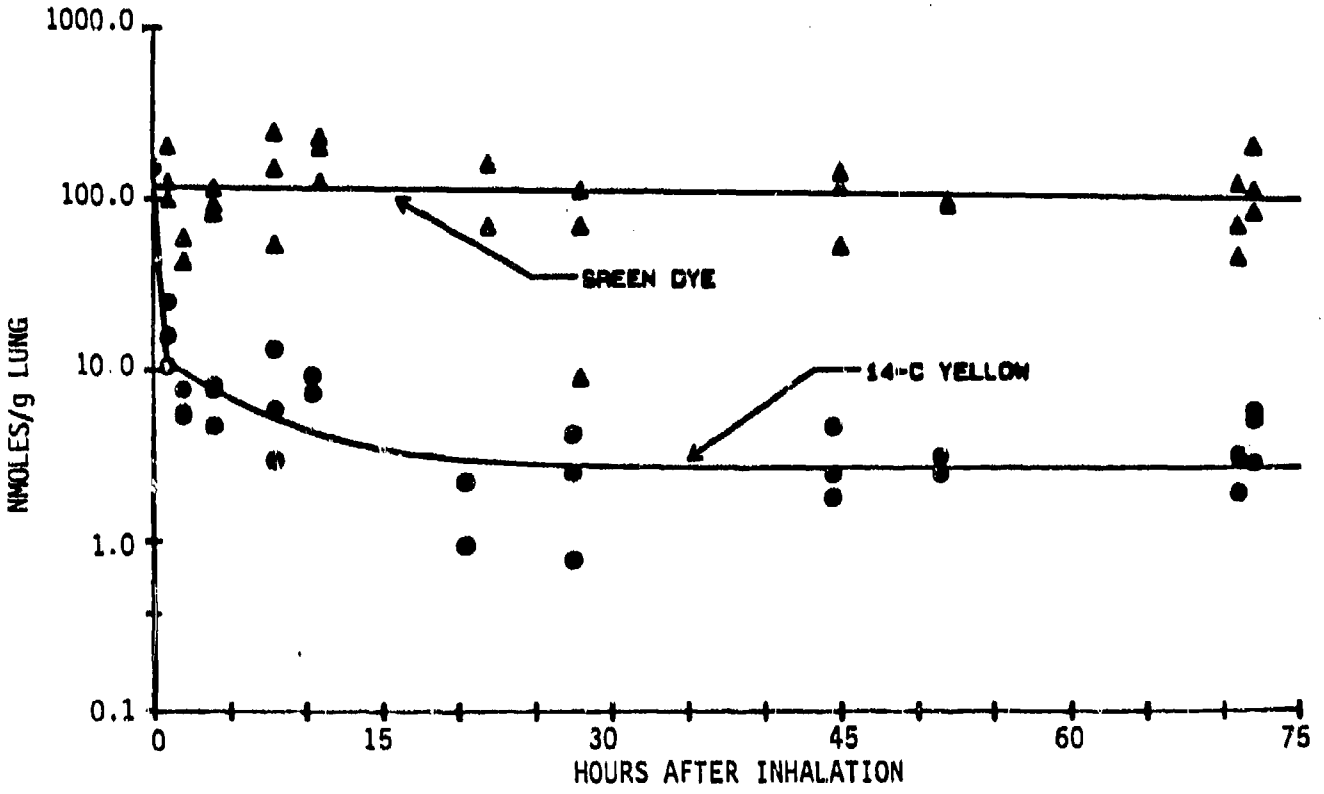
[a]Percent of total inhaled material that deposited in the respiratory tract.

The clearance of SG is compared to that of SY in Figure 12. The data are expressed as nmoles of dye/g lung. The datum point at t = 0 for SY is for comparison only and is based upon the assumption that none of the SY deposited in the lungs during the exposure was cleared from the lungs during the exposure. This number is based on the value for deposition of ^{14}C-SY in the lungs and bronchi calculated in Table 9. As is shown in Figure 12, SY is rapidly cleared from the lungs, while the SG is cleared slowly. Since neither dye is appreciably water soluble, and both are soluble in organic solvents, these results generate much speculation concerning the factors that govern solubility and clearance of material in the respiratory tract. One difference between the two dyes is that the SG is an anthraquinone and the SY is not. However, other investigators studying the toxicity of smoke composed of SY, SG, and disperse red, an anthraquinone, reported that only the SG was found in the lungs of rodents after 100 days exposure to the smoke mixture.[8] This suggests that solubility being equal, other factors, possibly molecular weight, might control the passage of molecules through the cell layers separating the alveoli and the blood capillaries.

Pathways for Excretion

Immediately after exposure 6 rats per exposure were placed in all-glass metabolism cages (Braintree Corp., Braintree, Mass.) for collection of excreta. Food and water were supplied. Urine and feces were collected separately in glass containers placed on dry ice. Expired air was drawn from each cage by means of a vacuum pump at a rate of 500 mL/min. Expired air from each cage was passed through a trap containing 200 mL of 1 M KOH for collection of any $^{14}CO_2$ that might result from the metabolism of ^{14}C-SY.

Figure 12. Comparison of the retention of ^{14}C-SY equivalents and SG equivalent in the lungs of rats exposed to a combination of ^{14}C-SY and SG. The data are presented as nmoles of dye/g lung. The datum point for SY at t - 0 is hypothetical and assumes that no clearance of SY occurred during the 60 min exposure. This point was reconstructed from the total respiratory tract burden of ^{14}C reported in Table 9, assuming that 14 percent of this material deposited in the lungs and bronchi.



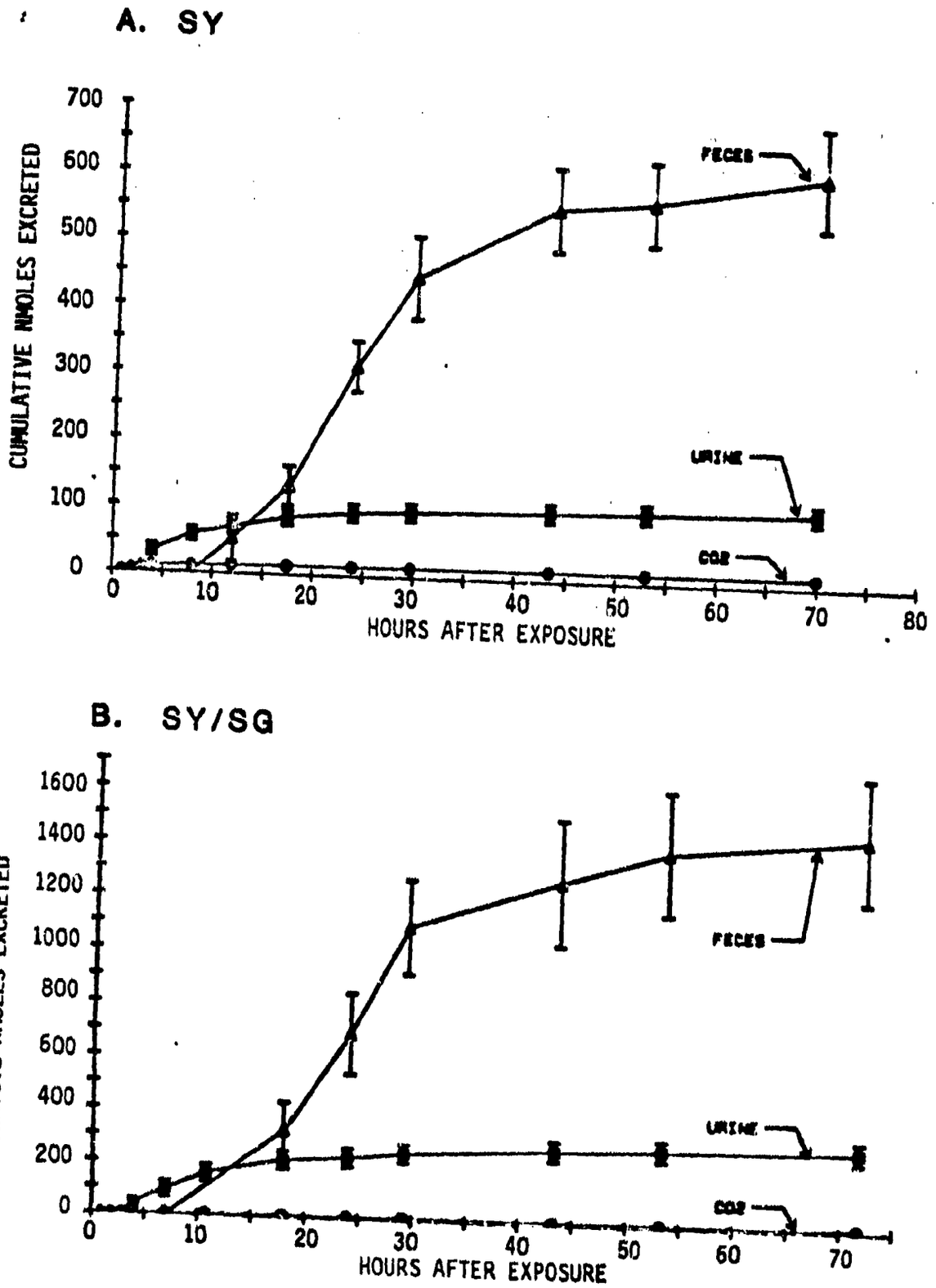
Urine and feces receptacles were changed at 4, 8, 12, 18, 24, 32, 44, 56, and 70 hr after administration of ^{14}C-SY. Traps of 1 M KOH were sampled every hour for the first 4 hr and then sampled and changed at the time of the other excreta collections. At 70 hr after exposure, rats were removed from the metabolism cages. Rats were sacrificed with an IP injection of T61 euthanasia solution, depelted, and necropsied as described above.

Urine, feces, and expired air samples were analyzed to quantitate the total radioactivity eliminated. Portions of urine (250 uL) and 0.5 mL samples of each KOH trap were added directly to liquid scintillation vials containing 10 mL of Ready-Solve (Beckman Instruments, Inc.). Fecal samples were digested by the addition of 1 to 5 mL of 1 percent Triton-X-100 in deionized water, and 500 mg portions of the digestate were oxidized as described above. Radioactivity was quantitated by analysis in a liquid scintillation spectrometer as described above.

Both the entire pelt and the remaining carcass were digested separately in TEAH at room temperature. Triplicate 500 mg samples were weighed and processed for radioanalysis as described above. Tissues taken at necropsy were also processed as described above.

The cumulative excretion of ^{14}C in urine or feces after exposure to SY alone or in combination with SG is presented in Figure 13 (A-B). As indicated, most of the ^{14}C was excreted in the feces. One potential pathway for fecal excretion of ^{14}C is the absorption of ^{14}C-SY into the blood from the lungs, uptake by the liver and excretion in bile. This hypothesis is supported by the fact that other investigators have found that solvent yellow is extensively excreted in the bile.[5] Alternatively, the SY deposited in the upper respiratory tract may have been cleared to the gastrointestinal

Figure 13. Cumulative excretion of nmoles of ^{14}C-SY equivalents in urine and feces or exhaled as $^{14}CO_2$ after exposure to (A) SY or to (B) SY in combination with SG.



tract via mucociliary clearance, and the majority of this cleared material might have been excreted in the feces.

Data for excretion of ^{14}C in urine or feces obtained for each rat exposed to SY alone or a combination of SY and SG were expressed as a fraction of the total amount excreted by each route with time after exposure. Data for all rats for both exposures were pooled and were expressed as the fraction remaining to be excreted in urine or feces as a function of time. A single-component negative exponential function was fit to each set of data by non-linear least-squares regression analysis (BMDP, University of California, 1979).

$$F(t) = e^{-kt},$$

where F(t) is the fraction yet to be excreted and
k is the apparent rate constant for elimination in hr^{-1}. Half-times for excretion of ^{14}C were determined as:

$$T_{1/2} = 0.693/k,$$

where k is as described above.

Data obtained for all rats for each exposure were also expressed as a function of time and analyzed separately. Apparent rate constants and half-times for elimination obtained are presented in Table 10. When the residual sum of squares obtained for each exposure concentration and that for all exposure concentrations combined were compared using a generalized F statistic,[9] there were no differences in the rates of excretion of ^{14}C in

TABLE 10. Apparent rate constants and half-times for excretion of ^{14}C-SY equivalents in urine or feces after exposure to SY aerosols alone or in combination with SG.

	Pathway for Excretion			
	Urine		Feces	
Exposure	Rate Constant[a] (hr^{-1})	$T_{1/2}$[b] (hr)	Rate Constant[a] (hr^{-1})	$T_{1/2}$[b] (hr)
SY Alone	0.070 ± 0.002[c]	10	0.051 ± 0.003	14
SY/SG	0.069 ± 0.002	10	0.047 ± 0.003	15

[a]Rate constants determined from a non-linear least squares regression analysis of the data using the function:

$$F(t) = e^{-Kt},$$

where F(t) is the fraction yet to be excreted and K is the apparent rate constant for elimination in hr^{-1}.

[b]Half-time, $T_{1/2}$, in hr was calculated from:

$$T_{1/2} = .693/K,$$

where K is described above.

[c]Parameter estimates ± SD, n = 6.

urine or feces as a function of exposure to SY alone or SY in combination with SG.

The half-times for excretion of ^{14}C suggest that ^{14}C is rapidly excreted from the body. This observation agrees with the observed rapid clearance of ^{14}C from tissues, and suggests that ^{14}C-SY equivalents are not retained in any tissues examined to any appreciable extent.

Table 11 summarizes the distribution of ^{14}C in tissues and excreta of rats at 70 hr after exposure to SY alone or to SY combined with SG. Data are expressed both as nmoles of ^{14}C-SY equivalents and percent of total ^{14}C deposited. A one-way analysis of variance indicated that a significantly higher percent of the total dose was exhaled as $^{14}CO_2$ in rats exposed to a combination of SY/SG compared to rats exposed to SY alone. However, as seen in Table 11, exhaled $^{14}CO_2$ is a very small fraction (< 2 percent) of the total amount deposited. At the end of 70 hr approximately 10 percent of the dose remained in the body. This material was found in tissues, in the carcass, and associated with the pelt.

SEPARATION OF ^{14}C-SY FROM METABOLITES

Tissue Preparation

A flow chart describing methods used for separation of unbound ^{14}C from tissue components and feces can be found in Figure 14. A similar method was used to separate ^{14}C-SY from water soluble metabolites in the urine. Briefly, samples (0.5-1 g) of lung, liver or kidney of rats sacrificed at 1 hr after exposure to SY alone or a combination of SY and SG, were weighed and homogenized in 1 mL of deionized water. The homogenate was transferred to a centrifuge tube, extracted for 5 min with 2 mL of acetonitrile, and

TABLE 11. Distribution of ^{14}C-SY equivalents in tissues and excreta (cumulative) at 70 hr exposure to SY alone or to SY combined with SG.[a]

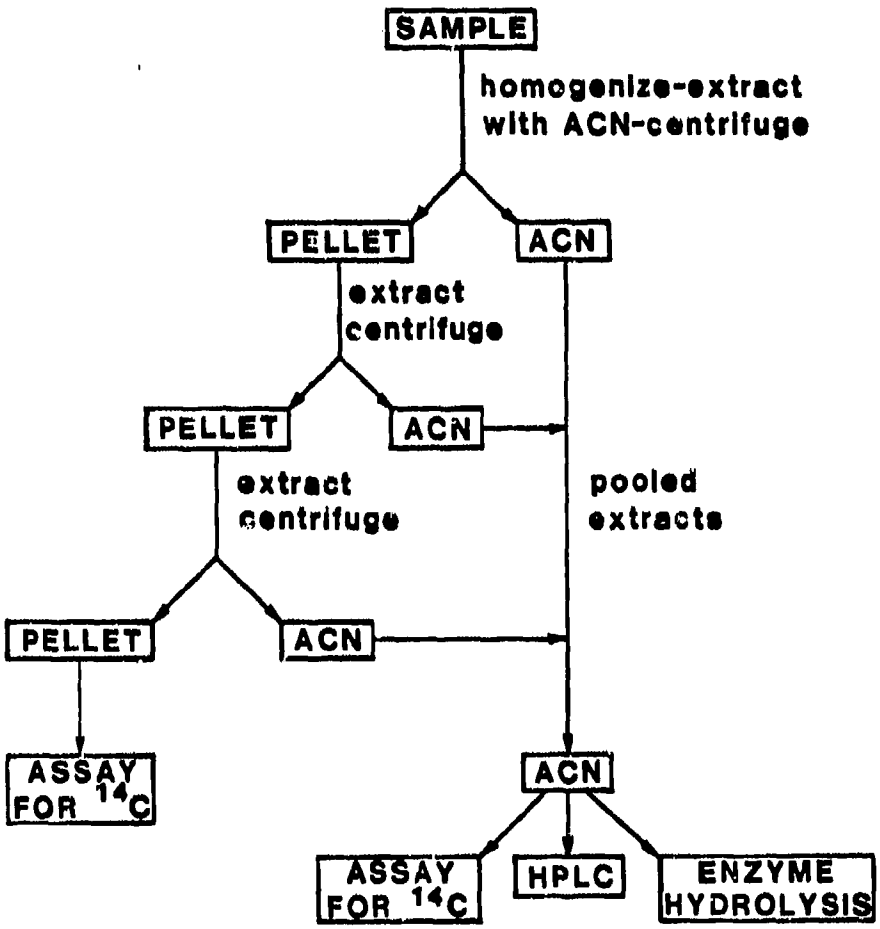
Exposure	Feces		Urine		Exhaled CO_2		Body[b]	
	nmoles	percent	nmoles	percent	nmoles	percent	nmoles	percent
43 µg SY/L	610 ± 75	77 ± 9	110 ± 14	14 ± 2	14 ± 1	1.8 ± 0.1[c]	61 ± 11	8 ± 1
93 µg SY/L + 154 µg SG/L	1460 ± 230	73 ± 12	290 ± 40	15 ± 2	10 ± 3	0.5 ± 0.2[d]	230 ± 45	12 ± 2

[a]Values are $\bar{X} \pm SE$, n = 6. The data are expressed as nmoles of ^{14}C equivalents or as the percent of the total recovered material that was excreted or remained in the body.

[b]Body includes pelt, carcass, and tissues.

[c,d]Values with different letters are significantly different using a one-way analysis of variance at a level of 0.05.

Figure 14. Diagram of the methods for separation of ^{14}C-SY and unbound metabolites from tissues or excreta from rats exposed to SY alone or to SY in combination with SG.



centrifuged. The supernatant was removed and the extraction procedure was repeated twice. The pellet was digested in TEAH and analyzed for ^{14}C. A portion (100 µL) of the pooled supernatant was analyzed for total ^{14}C and the remainder was divided into two samples. Both samples were evaporated to dryness at room temperature under a stream of nitrogen.

Feces excreted by each rat over the period of 24 to 48 hr were pooled, and a 1 g sample was weighed and extracted as described above. Urine samples for each rat collected during the first 24 hr were also pooled and treated as described above, with the exception that they were extracted with ethyl acetate instead of acetonitrile. The ethyl acetate layers obtained from each extraction were pooled, analyzed, and dried as described above. The remaining aqueous layer was analyzed for ^{14}C.

To determine the amount of ^{14}C that was associated with unmetabolized SY compared to more polar metabolites, one portion of the pooled extract from urine, feces, or tissues from each rat was analyzed by HPLC using a reverse phase analytical column (RP-C18; Alltech) and isocratic elution conditions of 90 percent acetonitrile/10 percent H_2O, with a flow rate of 1 mL/min. Fractions of the effluent were collected at 30 sec intervals. The retention time of purified SY standards chromatographed under identical conditions was determined by monitoring the absorption of the effluent at 435 nm.

Chromatography profiles of ACN extracts of lung, liver, kidney, and feces, and ethyl acetate extracts of urine are shown in Figures 15 and 16. Most of the ^{14}C in extracts of lung and liver chromatographed with a retention time identical to that of pure SY. In feces, kidney and urine samples, the fraction of ^{14}C that co-eluted with pure SY was much smaller, as determined by comparing the ^{14}C eluting with a retention time of 5.5 to 6 min to the total ^{14}C eluted.

Figure 15. Profiles of ^{14}C eluted by high pressure liquid chromatography of extracts of (A) lungs, (B) livers, or (C) kidneys of rats sacrificed at 1 hr after exposure to SY or SY combined with SG. The retention time of pure SY is indicated.

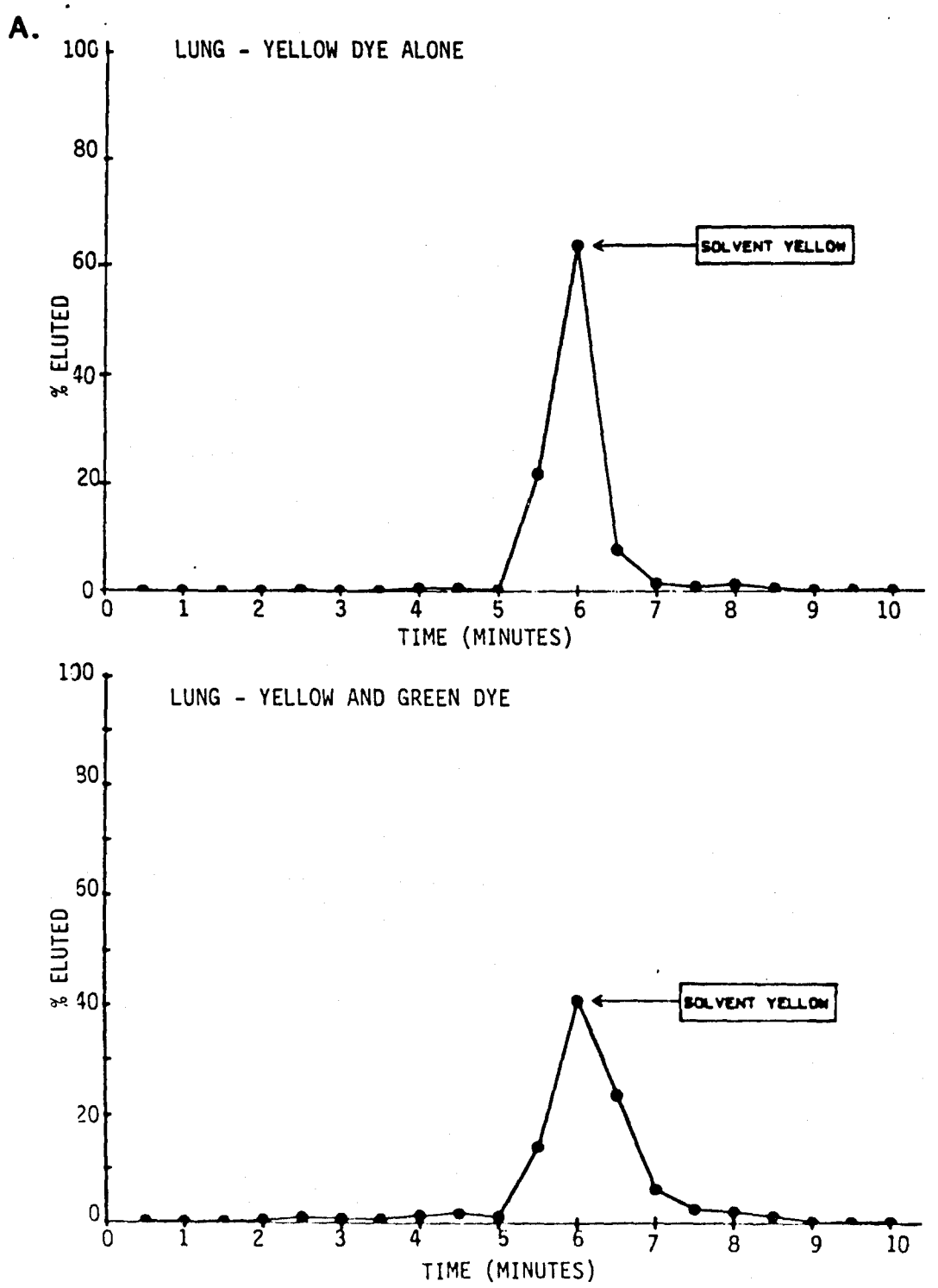
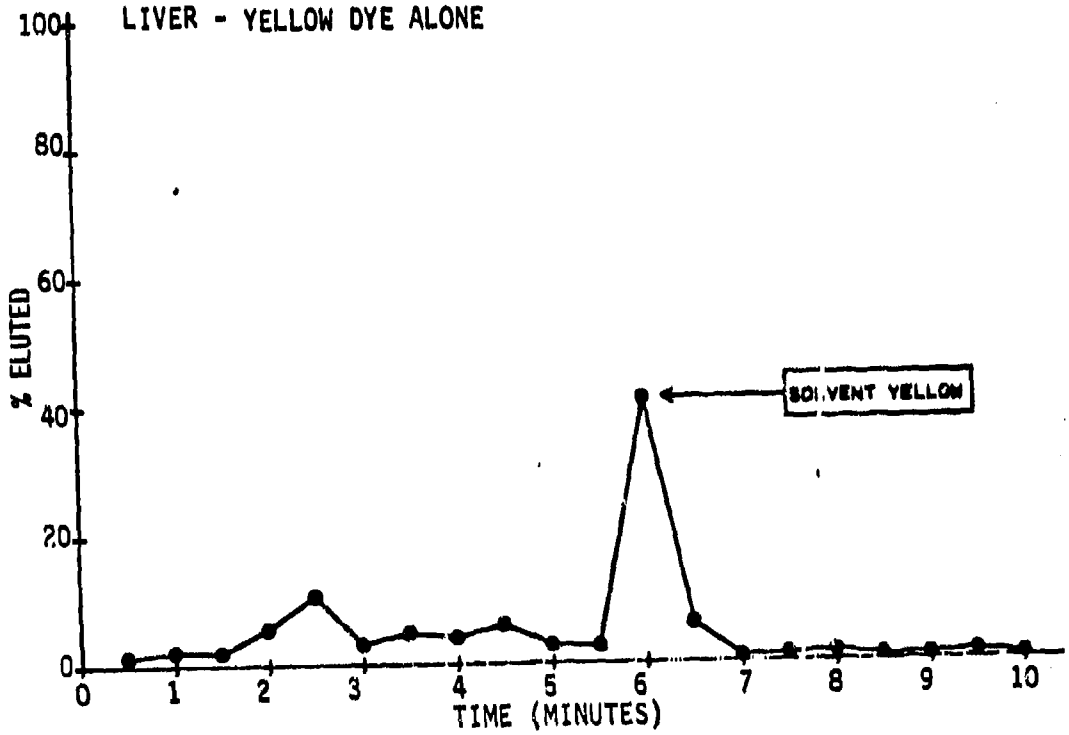


Figure 15.

B.


LIVER - YELLOW DYE ALONE
100
80
60
40
20
0
% ELUTED
SOLVENT YELLOW
0 1 2 3 4 5 6 7 8 9 10
TIME (MINUTES)

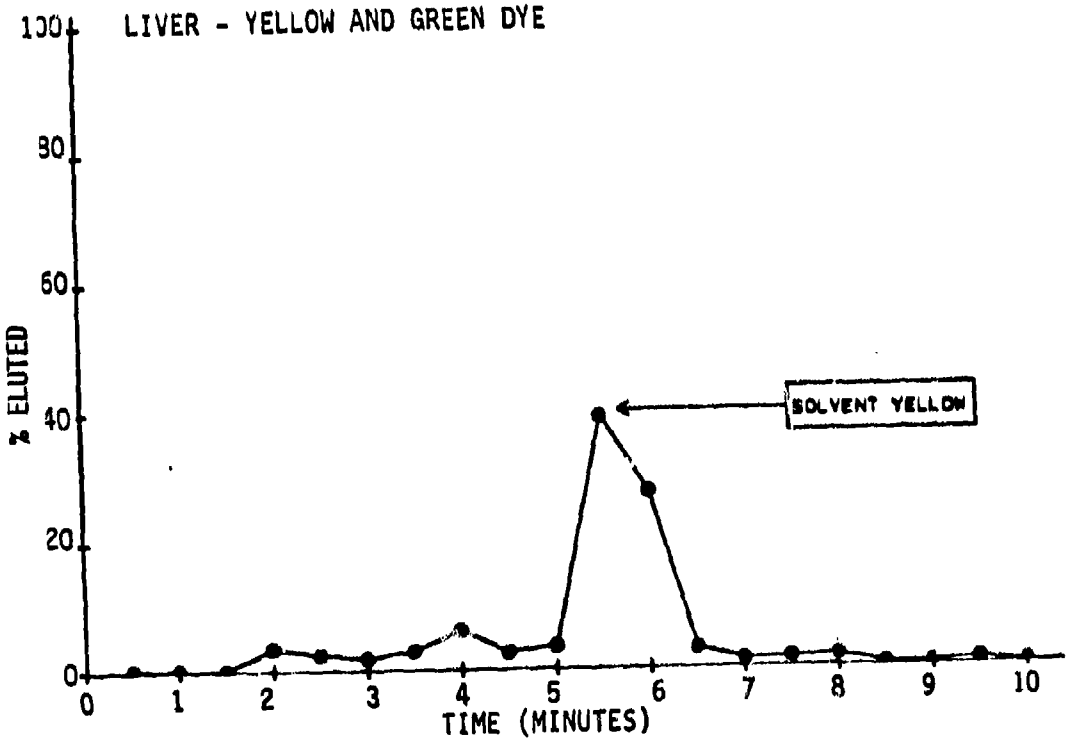
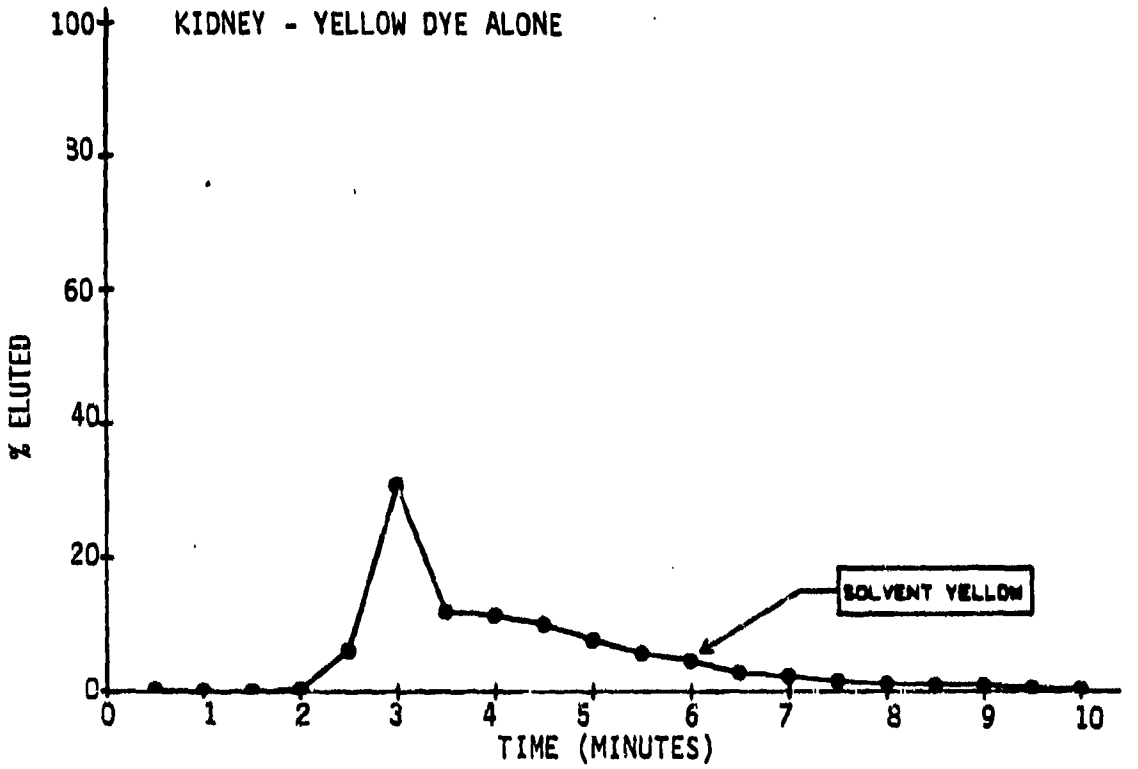

LIVER - YELLOW AND GREEN DYE
100
80
60
40
20
0
% ELUTED
SOLVENT YELLOW
0 1 2 3 4 5 6 7 8 9 10
TIME (MINUTES)

Figure 15.

C.


KIDNEY - YELLOW DYE ALONE
100
80
60
40
20
0
% ELUTED
SOLVENT YELLOW
0 1 2 3 4 5 6 7 8 9 10
TIME (MINUTES)

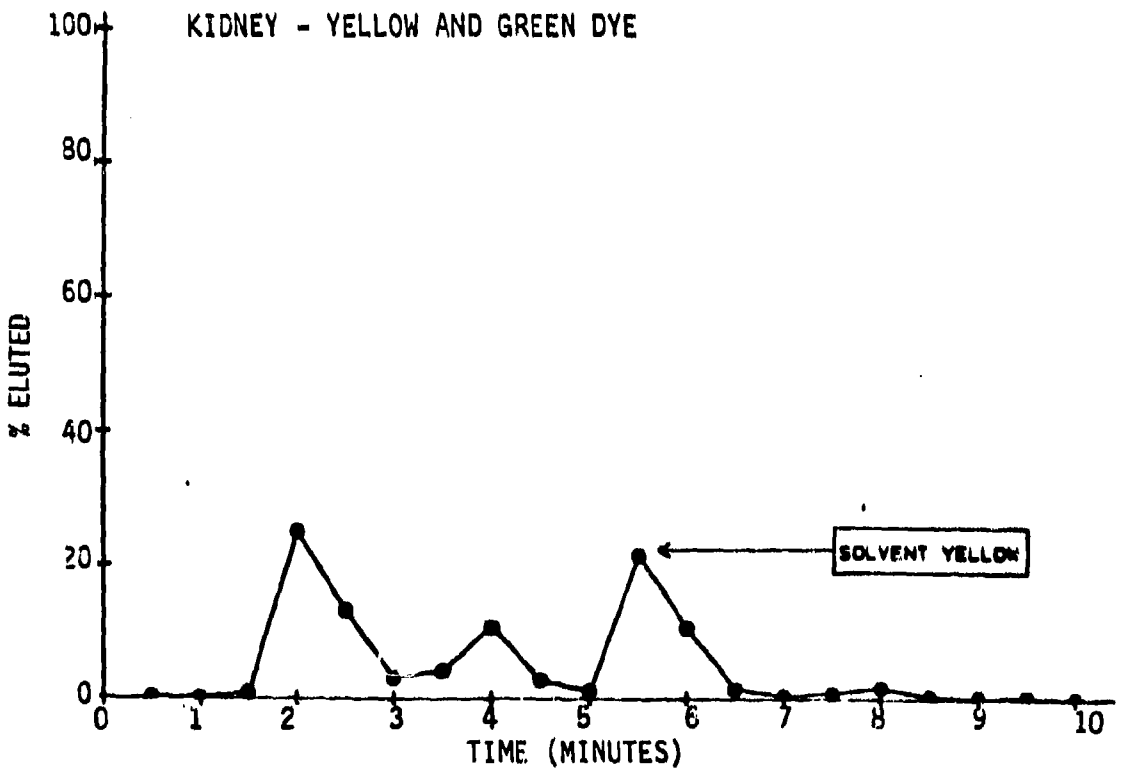
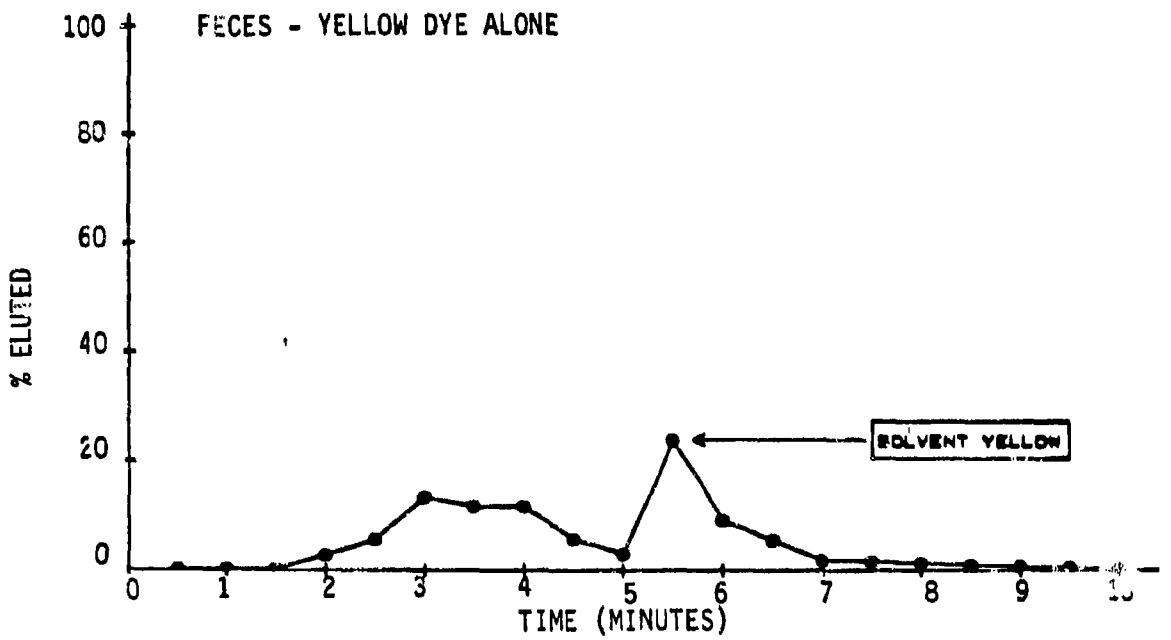

KIDNEY - YELLOW AND GREEN DYE
100
80
60
40
20
0
% ELUTED
SOLVENT YELLOW
0 1 2 3 4 5 6 7 8 9 10
TIME (MINUTES)

Figure 16. Profiles of ^{14}C eluted by high pressure liquid chromatography of extracts of (A) feces collected from 24 to 48 hr or (B) urine collected during the first 24 hr after exposure to SY alone or SY combined with SG. The retention time of pure SY is indicated.

A.



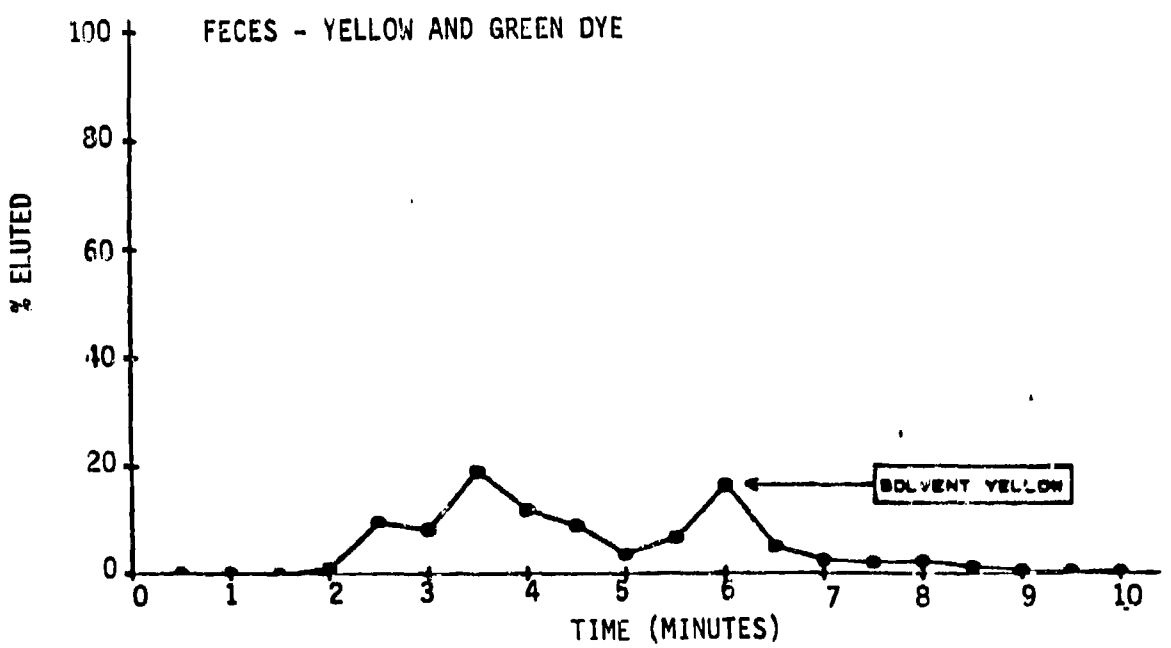
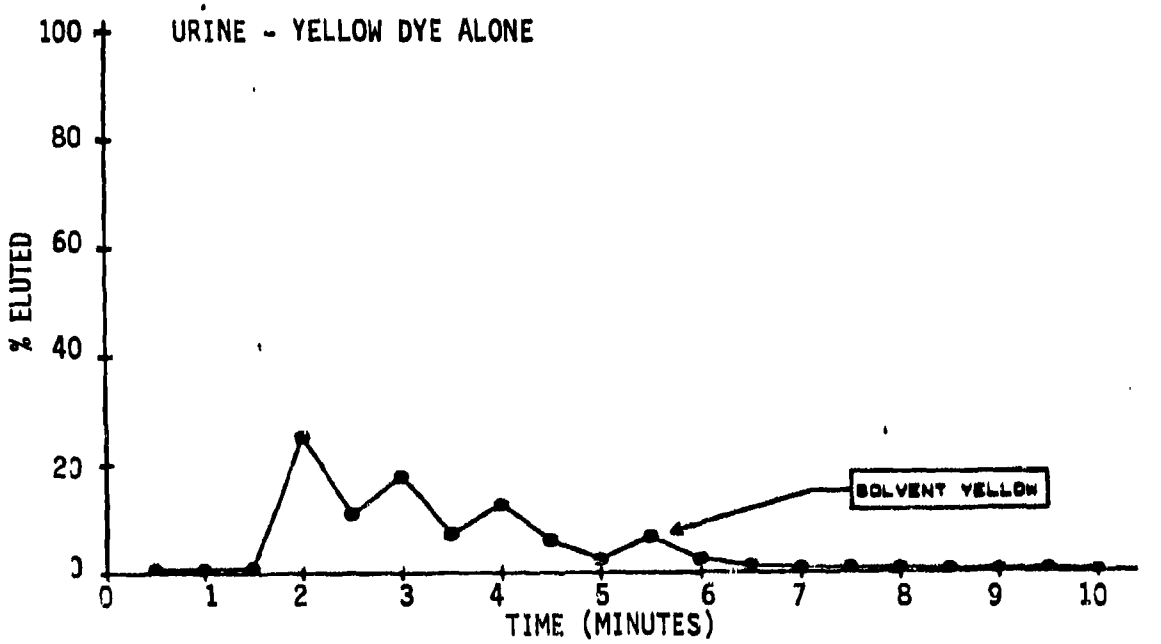


Figure 16.

B.


URINE - YELLOW DYE ALONE
100
80
60
40
20
0
% ELUTED
0
1
2
3
4
5
6
7
8
9
10
TIME (MINUTES)
SOLVENT YELLOW

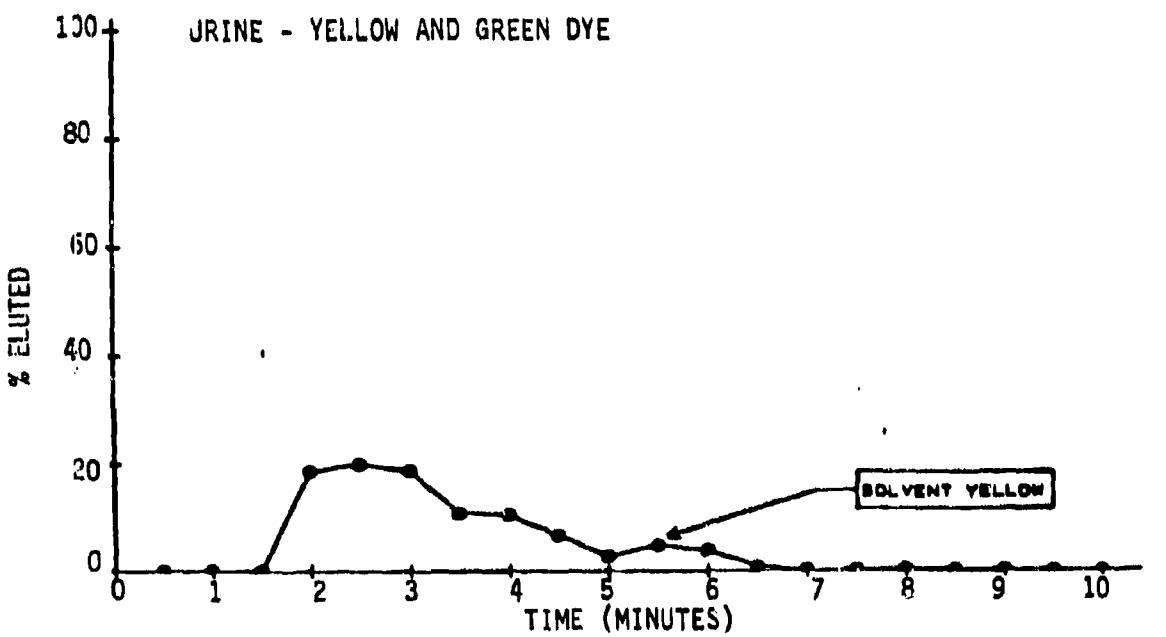

URINE - YELLOW AND GREEN DYE
100
80
60
40
20
0
% ELUTED
0
1
2
3
4
5
6
7
8
9
10
TIME (MINUTES)
SOLVENT YELLOW

Table 12 summarizes the extraction efficiency of ACN and ethyl acetate for ^{14}C-SY equivalents. Extraction efficiency of ACN was nearly 100 percent for lung and kidney samples, but was much lower for feces and liver samples. The unextracted material in liver and feces might be the result of SY being bound to tissue proteins or fecal material. The extraction efficiency of the organic solvent, ethyl acetate, for ^{14}C in urine was approximately 20 percent, suggesting that urine contained a large proportion of water soluble metabolites of SY. Table 12 also indicates the fraction of the total ^{14}C in the tissue or excreta samples that was associated with unmetabolized SY. We assumed that the extraction of SY in ethyl acetate or ACN was complete. We determined the percent of ^{14}C extracted that was associated with SY from the radioactivity profiles obtained by HPLC. Thus, ^{14}C-SY in the sample was a product of extraction efficiency for ^{14}C and the fraction of ^{14}C in the extract associated with unmetabolized SY. As seen in Table 12, the fraction of SY in the tissue and excreta samples ranged from 94 percent in the lung to 2-3 percent in the urine. Liver contained approximately one-third metabolized SY.

These results suggest that SY is extensively metabolized. It is most likely absorbed from the lung largely unaltered, and metabolized by the liver to subsequent urinary and biliary metabolites. Very little unaltered SY is excreted in the urine. The large fraction of unmetabolized SY in the kidney (10 to 35 percent) suggests that the kidney might also be the site of some metabolism of SY. This is the first time that the metabolism of SY has been documented. Other investigators have demonstrated that SY was extensively excreted in the bile[5] but they were unable to determine if SY was metabolized prior to excretion.

TABLE 12. Extraction efficiency of acetonitrile or ethyl acetate for total ^{14}C and for ^{14}C-SY from tissues or excreta of rats exposed to SY alone or to SY combined with SG.

Sample	Exposure	Extraction Efficiency[a] (%)	^{14}C-Associated with Unmetabolized SY in Extract[b] (%)	^{14}C-Associated with Unmetabolized SY in Sample[c] (%)
Lung	SY	99 ± 0.06	95 ± 0.8	94 ± 0.8
	SY + SG	99.5 ± 0.05	91 ± 0.4	91 ± 0.4
Liver	SY	96 ± 0.26	50 ± 9.3	48 ± 9
	SY + SG	97 ± 1.5	73 ± 2	71 ± 0.9
Kidney	SY	97.8 ± 0.14	16 ± 4	15 ± 4
	SY + SG	98.6 ± 0.20	34 ± 2	34 ± 2
Feces	SY	53 ± 5	40 ± 7	22 ± 6
	SY + SG	46 ± 6	31 ± 3	15 ± 3
Urine	SY	24 ± 1	13 ± 2	3 ± 0.4
	SY + SG	25 ± 5	12 ± 2	3 ± 1

[a]Extraction efficiency for acetonitrile (ACN) in lung, liver, kidney and feces samples. Represents material not associated with tissue pellet. Extraction efficiency for ethyl acetate (EA) in urine samples. Represents material removed from aqueous urine sample. Data represent means ± SE.

[b]^{14}C-SY in ACN or EA extracts quantitated by HPLC. Values are means ± SE of all profiles for a single tissue and exposure.

[c]^{14}C-SY in the entire tissue or excreta sample was the product of extraction efficiency and fraction of eluted ^{14}C associated with SY for each sample. Values are means ± SE of all determinations for a single sample and exposure.

To determine the presence of glucuronide or sulfate conjugates of SY metabolites, the second portion of the dried ACN extract of tissues and feces or a sample of unextracted urine was dissolved in sodium acetate buffer and divided into 3 samples. Samples were incubated with either β-glucuronidase or aryl sulfatase. Control samples were incubated with β-glucuronidase in the presence of saccarolactone, an inhibitor of β-glucuronidase. After incubation for 18 hr, at 37°C, the samples were extracted 3 times with ethyl acetate. Both the remaining aqueous layer and the ethyl acetate extract were analyzed for radioactivity.

Results indicated that pretreatment of samples with enzymes capable of cleaving glucuronide or sulfate conjugates did not increase the fraction of the radioactivity that could be extracted compared to untreated controls. Since cleavage of the glucuronide or sulfate bond would produce a less polar, or more readily extracted product, these results suggest that the polar metabolites of SY were probably not glucuronide or sulfate conjugates.

GASTROINTESTINAL ABSORPTION OF ^{14}C-SY

Inhaled material that is deposited in the respiratory tract can be cleared into the blood. Alternatively, material can be removed by mucociliary clearance to the pharynx and swallowed. Depending upon the extent of gastrointestinal absorption, this material can be absorbed from the gastrointestinal tract into the blood and contribute to the total absorbed dose. The purpose of this portion of the study was to determine what portion of the ^{14}C-SY transported to the gastrointestinal tract from the upper airways could be absorbed into the blood.

Three rats were given ^{14}C-SY (5 μCi; 655 nmoles/rat) suspended in 0.2 percent gelatin in saline by gavage. Three additional rats were given intratracheal instillations of ^{14}C-SY. Gavage and instillation volumes were 0.250 mL per rat. Between the gavage and the instillations, a sample of the dosing solution was taken and added to 250 mL of acetonitrile, and three 1-mL samples were analyzed for radioactivity.

After treatment, rats were placed in metabolism cages for collection of urine, feces, and expired $^{14}CO_2$ as described above. At the end of 94 hr, rats were sacrificed, and total radioactivity remaining in the body was determined as described above.

The dominant route of excretion of ^{14}C after either route of administration was via the feces, with 88 or 78 percent of the dose being excreted by this route after gavage or instillation, respectively. Significant quantities of ^{14}C were found in urine (8 percent of the dose after gavage or 15 percent after instillation). Very little radioactivity was exhaled as $^{14}CO_2$ (2 percent). From 1-2 percent of the dose remained in the tissues at 96 hr.

Gastrointestinal absorption of ^{14}C-SY was calculated as follows. We assumed 100 percent absorption for the instilled dose.

$$\text{Percent absorption} = 100 - F_g + F_i \left[U_g + CO_{2_g} / U_i + CO_{2_i}\right]$$

where F, U, and CO_2 were the percent of the dose in feces, urine or CO_2, respectively, after gavage (g) or instillation (i).
F_g is the percent of the dose excreted in feces after gavage. This material could represent both absorbed and unabsorbed ^{14}C. The next term, F_i,

represents the percent of the absorbed dose that would be expected to be excreted in feces.

The term $F_i[(U_g + CO_{2_g})/(U_i + CO_{2_i})]$ represents endogenous fecal excretion and indicates the proportion of the fecal ^{14}C that is due to ^{14}C absorbed from the GI tract and subsequently excreted in the feces.

$$\text{Percent absorbed} = 100 - 88 + 79[(8 + 2)/(15 + 2)] = 58 \text{ percent}$$

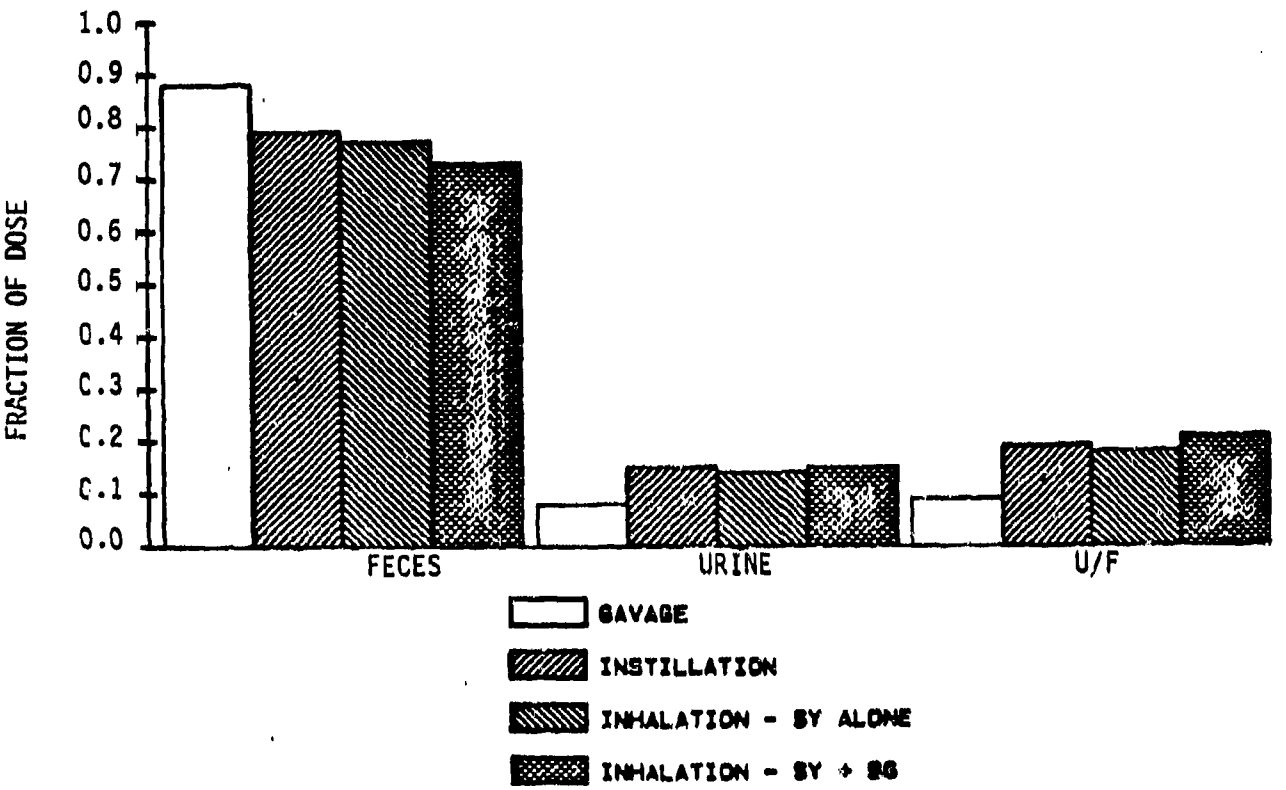
If one compares the percent of the total dose excreted in feces and urine after gavage, instillation, or inhalation (Figure 17), one sees that the ratio of ^{14}C in urine to feces is similar after inhalation or instillation, whereas a larger fraction of the ^{14}C is excreted in feces after gavage. This suggests that, as indicated above, gastrointestinal absorption of SY is incomplete.

SUMMARY AND CONCLUSIONS

In this study, rats were administered ^{14}C-SY by inhalation of pure SY aerosols or by inhalation of SY aerosols in combination with SG (a 0.6 ratio of yellow to green by weight). Endpoints measured were respiratory parameters and respiratory tract deposition, lung retention of SG and SY, distribution of ^{14}C-SY equivalents in tissues, metabolism of SY, and pathways for excretion of ^{14}C-SY equivalents.

Respiratory patterns were not affected by inhalation of either exposure atmosphere. Both total and regional fractional deposition were similar to deposition patterns seen with monodisperse aerosols of similar size. Our

Figure 17. Comparison of ^{14}C-SY equivalents excreted in urine and feces after inhalation of SY alone, SY in combination with SG, and after gavage or intratracheal instillation of ^{14}C-SY. U/F=ratio of counts in urine over feces.



results indicated that, following exposure, SY was rapidly cleared from the respiratory tract. SG, however, was much less rapidly cleared. Following absorption SY was extensively metabolized, most likely by the liver, but quite possibly also by the kidney. The major pathway for excretion of SY metabolites was via the feces, but some metabolites were also excreted in urine. Very little SY was metabolized to $^{14}CO_2$. By 72 hr after exposure, only 10 percent of the initial dose remained in the body. There was no effect of SG on the kinetics of distribution and elimination of SY. The rapid absorption, distribution, and excretion of SY compared to SG may explain, in part, the differences in lowest toxic concentration observed (> 250 mg/m^3 for SY and < 250 but ≥ 50 mg/m^3 for SG) seen in the toxicity studies conducted at our Institute.[10]

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GLOSSARY

Green component of yellow/green dye: green dye, solvent green, SG

Major compound in yellow dye: 2-(2'-quinolinyl)-1,3-indandione, QI

Major compound in green dye: 1,4-di-p-toluidinoanthraquinone, TA

Stock yellow dye: yellow dye, solvent yellow, SY

Stock yellow/green dye: yellow/green dye mix, SY/SG

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